

Probing ovulatory cycle shifts in women's preferences for men's behaviors

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Please note that the title has changed during the review process. Previous title was "*No evidence for ovulatory cycle shifts in women's preferences for men's behaviors in a pre-registered study*"

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Abstract

The existence of ovulatory cycle shifts in women's mate preferences has been discussed controversially. There is evidence that naturally cycling women in their fertile phase, compared to their luteal phase, evaluate specific behavioral cues in men as more attractive for sexual relationships. However, recent research has cast doubt on these findings. We addressed this debate in a large, pre-registered within-subject study including salivary hormone measures and luteinizing hormone tests. One-hundred-fifty-seven female participants rated natural videos of 70 men in dyadic intersexual interactions on sexual and long-term attractiveness. Multilevel comparisons across two ovulatory cycles indicated that women's mate preferences for men's behaviors did not shift across the cycle, neither for competitive, nor for courtship behavior. Hormone levels and relationship status did not affect these results. Hormonal mechanisms and implications for estrus theories are discussed.

Keywords: ovulatory cycle, mate preferences, steroid hormones, fertility, attractiveness

Introduction

Scientific interest in whether women experience systematic psychological changes across their ovulatory cycle has increased in recent years. A substantial amount of research indicates that women's sexual interests change across the ovulatory cycle. While cycle shifts in sexual desire appear robust, with higher levels of desire during women's fertile phase (e.g. Arslan, Schilling, Gerlach, & Penke, 2018; Grebe, Thompson, & Gangestad, 2016; Jones et al., 2018b; Roney & Simmons 2013; 2016), there is ongoing discussion whether there are changes in mate preferences as well. According to the good genes ovulatory shift hypothesis (henceforth *GGOSH*; Gangestad, Thornhill, & Garver-Apgar, 2005; Gangestad, Garver-Apgar, Simpson, & Cousins, 2007), women's mate preferences should differ according to the mating context: When fertile, women should prefer men with characteristics indicative of good genes for sexual relationships. These preferences should be absent in the luteal phase (i.e., between ovulation and menstrual onset) and when evaluating men for long-term relationships (as long-term bonding with these men can be costly, because they may be less willing to provide parental effort; Gangestad & Simpson, 2000).

Evidence for this hypothesis is mixed. Previous research has documented cycle shifts in women's mate preferences for several physical and behavioral traits (for an overview see Gildersleeve, Haselton, & Fales, 2014). However, changes in preferences for masculine faces, bodies and voices did not replicate in more recent studies (e.g. Jones et al., 2018a; Jünger, Kordsmeyer, Gerlach, & Penke, 2018a; Jünger et al., 2018b; Marcinkowska, Galbarczyk, & Jasienska, 2018; Muñoz-Reyes et al., 2014). Moreover, two meta-analyses have come to strikingly diverging conclusions on whether cycle effects exist or not (Gildersleeve et al., 2014; Wood, Kressel, Joshi, & Louie, 2014). Additionally, previously conducted studies have been criticized for potentially serious methodological problems, such as inappropriate sample sizes, using between-subject designs, lack of direct assessments of steroid hormones and not using luteinizing hormone (henceforth *LH*) tests for validating women's fertile phase (Blake,

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Dixson, O'Dean, & Denson 2016; Gangestad et al., 2016). In sum, to clarify the scientific discourse about the existence of ovulatory cycle shifts, there is strong need for adequately designed and powered replications.

Overview of the current study and hypotheses

In the current study, we set out to directly probe the GGOSH for men's behaviors, while overcoming previously reported methodological problems. In particular, we aimed to clarify a) whether there are preference shifts for men's behaviors across the ovulatory cycle, b) which hormonal mechanisms might potentially mediate these effects, and c) which moderators affect them.

Investigating ovulatory shifts in preferences for men's behaviors

Several studies report that women's preferences for men displaying behavioral dominance, confidence, and social presence change across the ovulatory cycle (Gangestad, Simpson, Cousins, Garver-Apgar, & Christensen, 2004; Gangestad et al., 2007; Lukaszewski & Roney, 2009). Moreover, previous research has also reported changes in women's flirting behavior and behavioral engagement with men displaying purported markers of genetic fitness (Cantú et al., 2014; Flowe, Swords, & Rockey, 2012). Women do also seem to show preferences for flirtatious facial movement in the fertile phase of the ovulatory cycle (Morrison, Clark, Gralewski, Campbell, & Penton-Voak, 2010). Yet, there is a lack of well-powered replications investigating preference shifts for men's behavior (Jones, Hahn, & DeBruine, in press). Therefore, we decided to investigate cycle shifts in preferences for men's flirting behavior and dominance-related cues found in such behavior. Flirting behavior was suggested to function to exaggerate one's qualities as a mate (Back, Penke, Schmukle, Sachse, Borkenau, & Asendorpf, 2011). Behavioral attractiveness can be seen as men's efforts to appeal attractive towards women in a more subtle and indirect manner than flirting behavior. Self-display behaviors have been seen as an attempt to impress the conversation partner, are correlated with higher testosterone levels in men and appear to index courtship-like behavior

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(Roney, Mahler, Maestripieri, 2003; Roney, Lukaszewski, & Simmons, 2007). The amount of eye contact (henceforth *gazes*) has been reported to be an indicator for social presence, a behavioral display for which women's mate preferences may change across the ovulatory cycle (Gangestad et al., 2004). Men's dominance, arrogance, assertiveness, confrontativeness, social respectedness, and likelihood to win a physical fight represent behaviors more directly related to intrasexual competitiveness and social presence, behaviors for which women's mate preferences had been reported to change across the cycle (Gangestad et al., 2004; 2007).

Following previous findings on ovulatory cycle shifts, we pre-registered the hypothesis that fertile women, as compared to when in their luteal phase, evaluate men's behaviors as more attractive for sexual relationships (Hypothesis 1). Moreover, women's mate preferences should shift across the cycle: When fertile, women should be more sexually attracted to men who show more overt flirting behavior, more self-displays, more direct gazes towards the women they were talking to, and more behavior that is consensually perceived as attractive (*behavioral attractiveness*; Hypothesis 2a). Furthermore, although not pre-registered¹, we expect comparable findings for behavioral cues of dominance, arrogance, assertiveness, confrontativeness, respectedness, and likelihood to win a physical fight. When evaluating long-term attractiveness, preference shifts should be absent or only weakly present (Hypothesis 3). We predict our findings to be robust when controlling for men's age, physical attractiveness, and voice attractiveness. We also state the alternative hypothesis that women's mate preferences for sexual relationships will not shift (Hypothesis 2b).

Hormones as mediators, relationship status as a moderator

Women's ovulatory cycle is regulated by shifts in hormone concentrations. While estradiol rises in women's fertile phase, it decreases during the luteal phase, but with a second smaller peak mid-luteal. Progesterone levels are usually lower in the fertile phase and higher

¹ We were asked to include those variables in the review process and agree that they better match the kinds of behaviors that were previously suggested to be evaluated differentially across the cycle.

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in the luteal phase. Therefore, cycle shifts in mate preferences should be mediated by natural changes in hormone levels: higher estradiol and lower progesterone (Hypothesis 4).

Moreover, the estradiol-to-progesterone-ratio (henceforth E/P) may be a superior predictor to estradiol or progesterone alone (Baird, Weinberg, Wilcox, McConnaughey, & Musey, 1991), with a higher E/P characterizing the fertile phase and a lower ratio characterizing the luteal phase. An important variable that might affect the strengths of ovulatory cycle shifts is women's relationship status. According to the dual mating hypothesis, women may receive fitness benefits when forming a relationship with a reliably investing man, while seeking good genes from other men through extra-pair sexual encounters (Pillsworth & Haselton, 2006). Since it remains unclear if singles also pursue different mating strategies across the cycle, we state two alternative hypotheses: Cycle shifts in preferences for short-term mates will be larger for partnered women than for single women (Hypotheses 5a), or, alternatively, relationship status will not affect the strengths of cycle shifts in preferences for short-term mates (Hypotheses 5b).

Material and Methods

Our hypotheses, the study design, the sampling and the analysis plan have been pre-registered online at the Open Science Framework (https://osf.io/egjwv/?view_only=91eb519f6d684637a47d1333c5f8856a), before any data on the women have been collected or analyzed. This pre-registration also contained further hypotheses that are not part of the present paper. Open data, analysis script, and instruction material is also provided. All participants signed a written consent form and the local ethics committee approved the study protocol (no. 144).

Participants and Recruitment

This sample is the same as in Jünger and colleagues 2018a and 2018b. Participants were recruited based on the inclusion criteria of other ovulatory cycle studies and had to fit to the following preregistered criteria: female, between 18 and 30 years old, naturally cycling

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(no hormonal contraception for at least three months, no expected switch to hormonal contraception during the study, no current pregnancy or breastfeeding, no birth-giving or breast-feeding during the previous three months, not taking hormone-based medication or anti-depressants). Additionally, they had to report that their ovulatory cycles had a regular length between 25 and 35 days during the last 3 months.

In total, we recruited 180 participants, of whom 23 could not be included in the final sample: Seventeen women who only attended the introductory session of the study dropped out before participation (six failed one of the inclusion criteria above, four quit the study, four did not respond to emails, three had scheduling problems). Another six dropped out during the study because they only completed the first testing session (four had scheduling problems, two did not respond to emails after the first session). One of the participants later reported to be 35 years old. We included her data for robustness checks because she met all other including criteria and had positive LH-tests. Excluding her data did not change the results. One-hundred-fifty-seven heterosexual female participants (aged 18-35 years, $M = 23.3$, $SD = 3.4$) finished all sessions and could therefore be included in further analyses. At the beginning of the study, 75 of these participants reported to be in a relationship, 82 reported to be single. Our sample largely exceeded the size required to achieve 80% power given a within-subject design and anticipated effects of moderate magnitude (Cohen's $d = 0.5$ with $N = 48$ for LH-test validated cycle phases and two testing sessions per participant, suggesting sufficient power to detect much smaller effect sizes in our study), as suggested by recent guidelines for sample sizes in ovulatory shift research (Gangestad et al., 2016). Upon completion of all sessions (see below), participants received a payment of 80€ or course credit.

Procedure

All participants took part in five individually scheduled sessions. In the first introductory session, participants received detailed information about the general procedure, duration of the study, and compensation. Furthermore, the experimenter explained the

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ovulation tests and checked the inclusion criteria. To count the days to the next ovulation and to plan the dates of the experimental sessions, cycle length as well as the dates of the last and the next menstrual onset were assessed. Finally, demographic data was collected.

Sessions two to five were computer-based testing sessions and took place once during the fertile phase and once during the luteal phase for two consecutive cycles per participant. To control for possible effects of diurnal changes in hormone levels, all sessions were scheduled in the second half of the day (mainly between 11.30 am and 6 pm). When arriving at the lab, participants first completed a screening questionnaire, assessing their eligibility and some control variables for saliva sampling (Schultheiss & Stanton, 2009). Saliva samples were collected via passive drool before the participants started the first rating task. Participants also completed other rating tasks and anthropometric data was collected between these tasks as part of a larger study (see pre-registration).

In the first testing session, participants saw a short preview video, presenting facial pictures of all men they were about to rate for one second each. Participants were then instructed to evaluate the men in the following videos, the actual stimulus material, according to their attractiveness as they perceived it “in that moment”, independent of their own current relationship status or general interest in other men, and to rate the attractiveness of the men by focusing only on the behavior as exhibited in the videos.

Video clips were presented in a randomized order using the experimental software Alfred (Treffenstaedt & Wiemann, 2018), which is based on the programming language Python (version 2.7). After watching each sequence, participants were to rate each individual man separately regarding sexual attractiveness (assessing short-term attraction) and attractiveness for long-term relationships. Ratings were done on eleven-point Likert scales from -5 (*extremely unattractive*) to +5 (*extremely attractive*), including zero as a neutral point. Definitions of sexual attractiveness and attractiveness for a long-term relationship were provided prior to the rating task:

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- a) *Sexually attractive*: Men that score high would be very attractive for a sexual relationship that can be short-lived and must not contain any other commitment. Men scoring low would be very unattractive for a sexual relationship.
- b) *Attractive for a long-term relationship*: Men that score high would be very attractive for a committed relationship with a long-term perspective. Men that score low would be very unattractive as a long-term partner.

After each session, the appointment for the next session was arranged individually based on the participant's ovulatory cycle.

Measures

Ovulatory cycle phase

Women's cycle phase was determined by the reverse cycle day method, based on the estimated day of the next menstrual onset (Gildersleeve, Haselton, Larson, & Pillsworth, 2012) and confirmed by highly sensitive (10 mIU/ml) urine ovulation test strips from Purbay®, which measure the luteinizing hormone (LH). These LH-tests had to be done at home at the estimated day of ovulation and the four days prior to that. The study investigated two ovulatory cycles in which each participant reported to the lab twice: Once while being fertile (at the days immediately preceding ovulation, usually reverse cycle day 16-18, with reverse cycle day 16 as the most ideal date) and once when not fertile (during the luteal phase, after ovulation and prior to the next menstrual onset, usually reverse cycle day 4-11, with reverse cycle days 6 to 8 as the most ideal dates). Out of all participants who finished every session, 66 participants started with the first session in their luteal phase, 91 started in the fertile phase.

For the main cycle phase analyses, we excluded a total of 45 participants due to negative LH tests in both cycles, irregular ovulatory cycles, or inappropriate scheduling of testing sessions (see "Preliminary Analyses" for more details), resulting in $n = 112$ women.

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Of these participants, 46 started with the first session in their luteal phase, and 66 started fertile. However, all 157 women were included in the denoted robustness checks.

Hormone assessments

For hormone assays, we collected four saliva samples from each participant, one per testing session. Contamination of saliva samples was minimized by asking participants to abstain from eating, drinking (except plain water), smoking, chewing gum, or brushing teeth for at least one hour before each session. Samples were visually inspected for blood contamination and stored at -80°C directly after collection until shipment on dry ice to the Kirschbaum Lab at Technical University of Dresden, Germany, where estradiol, progesterone, testosterone and cortisol were assessed via liquid chromatography mass spectrometry (LCMS). In only 22% of the hormone samples estradiol levels could be detected at all by LCMS analysis. Therefore, all samples were reanalyzed using a highly sensitive 17β -estradiol enzyme immunoassay kit (IBL International, Hamburg, Germany). These latter estradiol values were used in subsequent analyses. We centered all hormone values on their subject-specific means and scaled them afterwards (i.e. divided them by a constant), so that the majority of the distribution for each hormone varied from -0.5 to 0.5, to facilitate calculations in linear mixed models (e.g. Jones et al., 2018a; 2018b). This is a common procedure to isolate effects of within-subject changes in hormones, avoiding the influence of outliers on results and dealing with the non-normal distribution of hormone levels. It is also in line with our procedure in Jünger and colleagues (2018a; 2018b). Hormone levels were nearly normally distributed afterwards; a figure showing the distribution of hormone levels after this procedure can be found in the supplement (Figure S1). Importantly, this procedure did not change any findings compared to analyses with untransformed hormone values. The R code for this procedure can be found in the open script.

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Stimuli and behavioral ratings

Thirty seconds long sequences of videos of men in dyadic interactions, recorded in a study on sociosexuality (Penke & Asendorpf, 2008), were presented. We selected the videos of 70 men that were single at the time of the initial study out of a larger pool of 283 videos in total. For every video, a male participant was seated in a room with an attractive female confederate. They were instructed to get to know each other, while the experimenter left the room (see Penke & Asendorpf, 2008, for details). From each conversation, we took the sequence from 02:00 to 02:30 minutes to avoid the potential awkwardness of the first moments and ensure that the interaction was in full flow. The participants saw the conversation from a camera recording over the shoulder of the female confederate, so that they saw a frontal view of only the man in each interaction.

To get the behaviors of all men, videos were first rated by four independent, trained raters (two women, two men) that were unacquainted with the participants. Ratings were done using 7-point Likert scales for the 30-seconds sequences on the following behavioral dimensions: flirting behavior, self-displays, and behavioral attractiveness. Ratings were collected in two rounds, the first based on recordings from a side perspective, the second based on the frontal recordings that were used as stimuli in the present study. In both rounds, videos were presented with audio. Interrater agreement was high (side perspective: $\alpha = .84$ to $.88$; frontal perspective: $\alpha = .85$ to $.90$), thus ratings of all raters and both perspectives were aggregated.

Further ratings of the male behavior were collected separately later on. The following dimensions were rated: dominance, arrogance, assertiveness, confrontativeness, social respectedness, and likelihood to win a physical fight. Each dimension was separately rated by ten independent raters (five women, five men) based on the 30-second videos, using 7-point Likert scales. Interrater agreement was high (dominance $\alpha = .88$, arrogance $\alpha = .71$,

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assertiveness $\alpha = .89$, confrontativeness $\alpha = .83$, social respectedness $\alpha = .89$, likelihood to win a physical fight $\alpha = .86$), thus ratings of all raters were aggregated for each dimension.

In addition, codings of the objective behavior male gazes (percentage of total amount of time the man looked the confederate directly in the face) were done with Noldus Observer by two trained research assistants. Intraclass correlations were high (.99), thus codings from both assistants were averaged. Additionally, as control variables, men's facial and vocal attractiveness were rated on 7-point Likert scales. For facial attractiveness, frontal face pictures with neutral facial expressions were rated by 15 independent undergraduate students. Interrater reliabilites were high ($\alpha = .91$), so ratings were aggregated after z-standardization. For vocal attractiveness, voice recordings (counting from 1 to 10) were rated by six trained research assistants and ratings were aggregated afterwards ($\alpha = .80$). Behaviors varied substantially between the videos, descriptive values for all can be found in the supplement. More details about the rating and coding procedures can be found in Penke and Asendorpf (2008).

Statistical analyses

In our preregistration, we explicitly stated our hypotheses, methods, recruitment strategy and stopping rule. However, our preregistration was not fully explicit about our statistical analyses. Hence, we decided to run a number of robustness checks, which consist of analyses combining various reasonable analytical decisions. To substantiate that these choices are reasonable, we based them on previously published studies investigating cycle shifts in mate preferences or on suggestions we received during the review process. As described in our preregistration, all data analyses were done using multilevel modelling. Details can be found in the open script.

Results

Preliminary Analyses

First we checked how many of the participants' ovulatory cycles had positive LH tests (showing a LH surge) in the calculated fertile phase to detect non-ovulatory cycles. Twelve participants reported negative LH test results for both investigated cycles, nine reported negative LH tests results for one cycle. In total, LH tests in 33 of all 314 cycles (10.5%) were negative. Next, we counted how many cycles were reported as being irregular, that is, where days of the testing sessions deviated from the prior defined phase of appropriate testing days by more than three days (see section Ovulatory Cycle Phase). Eight women reported irregular cycles in both investigated cycles, 32 reported one cycle being irregular, resulting in 48 out of 314 (15.3%) cycles being irregular (despite all participants reporting having regular ovulatory cycles in the introductory session prior to the testing sessions). Additionally, we checked the temporal relationship between the reported day of LH surge and the date of scheduled testing session. Because ovulation usually occurs within 24-36 hours after the observed LH surge, testing sessions that were scheduled more than two days after the surge might have already been in the early luteal phase. Out of the 281 cycles for which an LH surge was observed, thirteen (4.63%) purportedly fertile phase sessions were scheduled three or four days after the LH surge. Therefore, 268 (95.37%) were scheduled within an appropriate range of three days before to two days after the LH surge (in total: $M = -0.12$, $SD = 1.39$ days in relation to the day of the observed LH surge). A histogram showing the distribution of days of fertile phase testing sessions relative to the observed LH surge can be found in the supplement (Figure S2). Participants with irregular cycles, negative LH-tests or the risk of early luteal phase instead of fertile phase testing session were excluded in the main analyses (as cycle phase estimates based on LH have a much higher validity than estimates based on backward counting alone, e.g. Blake et al., 2016; Gangestad et al., 2016), but included in denoted robustness checks in the supplement.

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Main analyses: Cycle shifts in women's attraction and mate preferences

We first tested for possible ovulatory cycle shifts in women's attractiveness ratings for men's behavior in general (Hypothesis 1). For multilevel analyses with attractiveness rating as dependent variable (Model 1 with sexual attractiveness, Model 2 with long-term attractiveness), female raters and male stimuli were treated as random effects. Women's cycle phase (0 = luteal phase, 1 = fertile phase) was treated as a fixed effect. We additionally let participant's slopes vary systematically across cycle phase by modeling cycle phase as a random slope. Both models showed a significant cycle shift in women's attraction: When fertile, ratings for sexual attractiveness were higher than in the luteal phase of the ovulatory cycle ($\gamma = 0.10$, $SE = 0.05$, $t = 2.13$, $p = .035$, $95\%CI = [0.01; 0.20]$), supporting Hypothesis 1. Similar results were found for the long-term attractiveness ratings ($\gamma = 0.10$, $SE = 0.05$, $t = 2.05$, $p = .042$, $95\%CI = [0.00; 0.19]$). When women were fertile, the attractiveness ratings of men's flirting behavior increased compared to the ratings in the luteal phase. As robustness checks, we repeated these analyses with E/P as a predictor rather than cycle phase, using the dataset of $N = 157$ women, not including random slopes, resulting in seven additional models for sexual and long-term attractiveness each. Significant effects were observed in only two out of seven models, all with cycle phase as a predictor (none of the models with E/P as predictor were significant). Details can be found in the supplementary material (Tables S1 – S7).

Next, we analyzed whether women's mate preferences for specific behaviors changed across the cycle (Hypotheses 2a, 2b and 3). Deviating from our preregistration based on suggestions resulting from the review process, we ran a factor analysis for the different behavioral tactics, because some of them correlated rather strongly (Table S8). An exploratory factor analysis with oblimin rotation yielded two factors: Factor 1 (labelled "competitiveness") with positive loadings of dominance, confrontativeness, assertiveness, likeliness to win a fight, self-display behavior, arrogance, and respectedness, Factor 2

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(labelled “courtship”) with positive loadings of flirting behavior, behavioral attractiveness, respectedness, gazes, and negative loadings of arrogance. Factor scores were saved for further computations, detailed factor loadings can be found in Table S9. Both factors were correlated ($r = .55$, $p < .001$) and predicted attractiveness ratings (*competitiveness*: sexual attractiveness: ($\gamma = 1.00$, $SE = 0.15$, $t = 6.83$, $p = < .001$, 95% CI = [0.71; 1.29]); long-term attractiveness: ($\gamma = 0.86$, $SE = 0.15$, $t = 5.73$, $p = < .001$, 95% CI = [0.57; 1.16]); *courtship*: sexual attractiveness: ($\gamma = 1.44$, $SE = 0.10$, $t = 15.06$, $p = < .001$, 95% CI = [1.25; 1.63]); long-term attractiveness: ($\gamma = 1.40$, $SE = 0.09$, $t = 15.63$, $p = < .001$, 95% CI = [1.22; 1.58])). To decrease the length of our manuscript, we report all other results for the long-term attractiveness ratings in the supplementary material (Tables S24 – S30).

We computed multilevel models with women's cycle phase and either the competitiveness factor (Model 3) or the courtship factor (Model 4) as fixed effects. Female participants as well as male stimuli were treated as random effects and a random slope for cycle phase varying within participants was included. Results revealed no significant interactions of cycle phase and the behavioral factors, indicating that women's mate preferences for specific cues in men's behavior did not shift across the ovulatory cycle, contradicting Hypothesis 2a, but supporting alternative Hypothesis 2b. However, there were significant main effects for cycle phase and both factors on sexual attractiveness ratings (Table 1). The effects were comparable for long-term attractiveness ratings (Table S24), thus in line with Hypothesis 3. All results were virtually identical when controlling for men's age, physical attractiveness, and voice attractiveness (Tables S10, S27). However, men's age had a significant main effect on sexual and long-term attractiveness ratings in all courtship models, physical attractiveness on both ratings in all competitiveness models and vocal attractiveness in competitiveness models on sexual attractiveness only.

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Table 1

Multilevel regression analyses of attractiveness ratings as a function of cycle phase and the behavioral factors.

	γ	<i>SE</i>	<i>t</i>	<i>p</i>	95% <i>CI</i>
Competitiveness model					
Cycle phase	0.10	0.05	2.13	.035	[0.01; 0.20]
Competitiveness	1.01	0.15	6.83	<.001	[0.72; 1.30]
Cycle phase x competitiveness	0.02	0.02	0.80	.422	[-0.03; 0.07]
Courtship model					
Cycle phase	0.10	0.05	2.13	.035	[0.01; 0.20]
Courtship	1.44	0.10	14.95	<.001	[1.25; 1.63]
Cycle phase x courtship	0.02	0.02	0.65	.518	[-0.03; 0.07]

Note. Each model had 31,360 observations (each 112 participants x 4 test sessions x 70 stimuli).

Following our preregistration, we additionally computed separate models for all behavioral cues. None of these models revealed a significant interaction between cycle phase and cue. Details can be found in the supplement (Tables S11, S12, S29, S30). Again, we found some main effects for individual behavioral cues on sexual and long-term attractiveness: ratings were higher when flirting behavior, self-display behavior, behavioral attractiveness, assertiveness, confrontativeness, dominance, likeliness to win a fight, and respectedness were higher. There were no significant main effects for gazes or arrogance. All results were virtually identical when controlling for men's age, physical attractiveness, and voice attractiveness (Tables S13 – S15).

Hormonal mechanism potentially underlying preference shifts

To investigate possible effects of steroid hormones underlying cycle shifts in women's mate preferences for sexual attractiveness as dependent variable (Hypothesis 4), we entered E/P and either the competitiveness factor (Model 5) or the courtship factor (Model 6) as fixed effects to our multilevel model, female participants and male stimuli as random effects, and a random slope for E/P varying within participants. Results revealed no significant interaction of E/P and the behavioral factors, indicating that women's mate preferences for specific cues in men's behavior did not shift due to changes in E/P, contradicting Hypothesis 4. However, there were significant main effects for both factors on sexual attractiveness ratings (Table 2).

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The effects were comparable for long-term attractiveness ratings (Table S25), thus in line with Hypothesis 3. All results were virtually identical when controlling for men's age, physical attractiveness, and voice attractiveness (Tables S16, S30) or when adding estradiol and progesterone rather than E/P as predictor variables (Table S17).

Table 2

Multilevel regression analyses of attractiveness ratings as a function of E/P and the behavioral factors.

	γ	<i>SE</i>	<i>t</i>	<i>p</i>	95% <i>CI</i>
Competitiveness model					
E/P	0.02	0.05	0.30	.763	[-0.09; 0.12]
Competitiveness	1.01	0.15	6.91	<.001	[0.72; 1.29]
E/P x competitiveness	0.00	0.02	0.05	.959	[-0.04; 0.05]
Courtship model					
E/P	0.02	0.05	0.30	.763	[-0.09; 0.12]
Courtship	1.44	0.10	15.04	<.001	[1.25; 1.63]
E/P x courtship	0.01	0.02	0.51	.610	[-0.03; 0.06]

Note. Each model had 27,300 observations (each 112 participants x 4 test sessions x 70 stimuli-missing values).

The role of women's relationship status for ovulatory cycle shifts

In order to analyze whether women's relationship status might moderate ovulatory cycle shifts (Hypothesis 5), we categorized all women as being in a relationship who reported to be in an open relationship, in a committed relationship, engaged, or married. However, results did not change when categorizing women who reported to be in an open relationship as singles instead. Relationship status changed for 13 women across the study; these cases were categorized according to their relationship status on the particular testing day. Two multilevel models (Model 7 with competitiveness, Model 8 with courtship as independent variables), both with women's cycle phase and their relationship status as fixed effects and female participants and male stimuli as random effects and random slope variations for cycle phase and relationship status varying within participants were included. Results showed significant main effects for competitiveness and courtship, in that ratings were generally higher when competitiveness or courtship were higher. Moreover, there was a significant interaction effect

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between competitiveness and relationship status in that women in relationships showed stronger preferences for competitiveness than singles. There were no other significant effects: neither the main effects of cycle phase or relationship status were significant, nor any interaction effect involving cycle phase (Table 3), indicating that women's mate preferences did not shift across the cycle and that relationship status does not moderate such shifts, supporting Hypothesis 5b, but not Hypothesis 5a. Results for long-term attractiveness revealed comparable results (Tables S26).

Table 3

Multilevel regression analyses of attractiveness ratings as a function of cycle phase, relationship status and the competitiveness factor or the courtship factor.

	γ	<i>SE</i>	<i>t</i>	<i>p</i>	95% <i>CI</i>
Competitiveness model					
Cycle phase	0.06	0.07	0.93	.355	[-0.07; 0.19]
Competitiveness	0.97	0.15	6.57	<.001	[0.68; 1.26]
Relationship status	-0.25	0.14	-1.79	.086	[-0.54; 0.02]
Cycle phase x competitiveness	0.02	0.03	0.64	.524	[-0.04; 0.09]
Cycle phase x relationship status	0.09	0.09	0.92	.360	[-0.10; 0.27]
Competitiveness x relationship status	0.07	0.03	2.03	.043	[0.00; 0.13]
Cycle phase x competitiveness x relationship status	-0.01	0.05	-0.15	.883	[-0.10; 0.09]
Courtship model					
Cycle phase	0.06	0.07	0.93	.355	[-0.07; 0.19]
Courtship	1.42	0.10	14.44	<.001	[1.22; 1.61]
Relationship status	-0.25	0.14	-1.79	.086	[-0.54; 0.02]
Cycle phase x courtship	0.02	0.03	0.54	.593	[-0.05; 0.09]
Cycle phase x relationship status	0.09	0.09	0.92	.360	[-0.10; 0.27]
Courtship x relationship status	0.06	0.04	1.75	.081	[-0.01; 0.13]
Cycle phase x courtship x relationship status	-0.01	0.05	-0.15	.878	[-0.11; 0.09]

Note. Each model had 31,360 observations (each 112 participants x 4 test sessions x 70 stimuli).

For robustness checks, we repeated our analyses with E/P as a predictor variable rather than cycle phase. None of the two-way or three-way interactions involving E/P were significant (Table S18). All results were virtually identical when controlling for men's age, physical attractiveness and voice attractiveness (Tables S19, S20).

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Additional robustness checks

We conducted further analyses to probe the robustness of our effects. To rule out that our results might have been caused by order effects of testing sessions, particularly participating in the first session when fertile (Suschinsky, Bossio, & Chivers, 2014), we controlled for initial cycle phase in our main analyses. Initial cycle phase affected attractiveness ratings, as they were higher when participants were tested first when fertile. However, all other results remained stable (Tables S21 – S23). We then repeated all main analyses with all recruited participants ($N = 157$). Results remained virtually identical and can be found in the supplement (Tables S31 – S37). In summary, the null results regarding cycle shifts in mate preferences remained robust across all checks.

Discussion

This study investigated ovulatory cycle shifts in women's mate preferences for men's behaviors. We included different cycle phase predictors, direct hormonal measures, relationship status as moderator variable, and a number of different behavioral cues. We did not observe compelling evidence for ovulatory cycle shifts in women's mate preferences, neither across different cycle phases, nor as predicted by hormone levels. Effects were not influenced by women's relationship status and remained non-significant across multiple robustness checks. Results did not differ considerably between sexual and long-term attractiveness ratings. Thus, the current study's results do not provide evidence for the GGOSH, contradicting previous findings for ovulatory cycle shifts for men's behaviors (Gangestad et al., 2004; 2007; Lukaszewski & Roney, 2009), but are in line with recent non-replications of cycle shifts in mate preferences for masculine faces, voices, and bodies (Jones et al., 2018a; Jünger et al., 2018a; 2018b; Marcinkowska et al., 2018). Although the effect sizes for preference shifts might be small, our study had enough power to detect even rather small effects, employing the largest sample size so far, a within-subject design with four testing sessions, and high cycle phase validity due to LH tests. Previous studies that have

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reported evidence for the GGOSH contain various issues in these regards that might have led to overestimation of effect sizes and false positive results. However, the fact that we did not find support for the GGOSH does not mean that preference shifts do not exist in general. For example, preference shifts for other cue domains (e.g. odor) might be robust and we do not know if preference shifts for behaviors only occur under specific conditions (e.g. male intrasexual competition) or only for specific women (e.g. personality differences in preference shifts, influences of partner attractiveness). Given that the current sample is the same as in Jünger et al., 2018a and 2018b, we cannot rule out that our reported null findings are sample specific and recommend replication in an independent sample.

Instead of preference shifts, shifts in women's general attraction to men were recently reported in the same data (Jünger et al., 2018a; 2018b). Here, we observed partial evidence for this effect in that ratings for sexual as well as for long-term attractiveness were higher in the fertile phase, compared to the luteal phase. However, this effect was not significant across all robustness checks and the lack of ties to hormone levels is a fairly significant limitation to this finding. Hence, we recommend independent replications, preferably in large, preregistered studies, to probe the robustness of this effect.

Further, future research should also aim to directly compare competing theories from the literature against the GGOSH (e.g. cycle shifts as vestigial by products of hormonal changes [Thornhill & Gangestad, 2015], motivational priority shifts [Roney, 2018], or the “spandrels hypothesis” of cycle shifts as a by-product of between-women hormonal differences [Havlíček, Cobey, Barrett, Klapilová, & Roberts, 2015]).

Limitations

We also note an important limitation that should be addressed in future research. Previous studies that found evidence for cycle phase shifts in preferences for men's behaviors focused more directly on behaviors related to dominance and social presence within an intrasexual competitive context. In contrast, we used videos of an intersexual courtship context. It is

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possible that the behaviors assessed in competitive contexts (e.g. Gangestad et al., 2004; 2007) were better indicators of good genes, because they implied a willingness to risk confrontations with other men, whereas flirting with women in the absence of same-sex rivals may not carry similar implications. However, social behaviors like dominance and social presence are somewhat stable across situations (Funder & Colvin, 1991; with an average reliability of .78 for dominance across situations). Hence, for example, a man who behaves dominantly in intrasexual competitive situations might also show more dominant behavior in flirting situations. Indeed, stimulus ratings showed a high inter-rater agreement for competitive behaviors and factor analysis yielded a competitiveness factor alongside a courtship factor, indicating that these behaviors could be consensually perceived from our stimulus material. Both factors had a significant effect on attractiveness ratings, suggesting that women do have preferences for men displaying more competitive and courtship behaviors even in the absence of a rival, though they seem not to shift across the cycle. Nevertheless, it remains unclear if preference shifts would have been observable if women had watched and evaluated an intrasexual competitive scene between two men rather than an intersexual courtship context.

Conclusion

In conclusion, in the largest study conducted so far investigating possible cycle shifts in women's mate preferences for men's behaviors, we did not observe shifting mate preferences across the cycle. As such, our findings are inconsistent with the GGOSH. It remains unclear whether women's general attraction to men shifts across the cycle and whether preference shifts for other cues are robust. Future studies combining rigorous methods and large sample sizes with precise preregistration will be crucial to further elucidate these issues.

Author contributions

JJ and LP developed the study concept and contributed to the study design. Testing and data collection were performed by JJ, who also performed data analysis and interpretation under the supervision of LP. JJ drafted the manuscript and TMG and LP provided critical revisions. All authors approved the final version of the manuscript for submission.

Declaration of Conflicting Interests

The authors declare that they have no conflicts of interests.

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Open practices statement

The study reported in this article was preregistered. The preregistration, data, analysis script and instruction materials have been made available at the Open Science Framework (https://osf.io/egjwv/?view_only=91eb519f6d684637a47d1333c5f8856a). However, we do not have consent to make the stimulus videos publicly available.

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