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Changes in preference for male faces during the menstrual cycle in a Spanish population

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Título: Cambios en la preferencia por rostros masculinos durante el ciclo menstrual en población española.

Resumen: Recientemente se ha postulado una controvertida hipótesis que propone la presencia de un periodo de estro en las mujeres, como ocurre en otros mamíferos. Ello implica que las mujeres en el óptimo de fertilidad del ciclo menstrual presenten comportamientos encaminados a maximizar la calidad genética de su descendencia. Diversas investigaciones sostienen esta hipótesis, al encontrar que las mujeres en la fase fértil prefieren hombres con rasgos que denotan mayor calidad fenotípica, como un mayor grado de masculinización o una mayor simetría. Nuestro objetivo ha sido testar experimentalmente en una población de 810 jóvenes españolas alguna de estas observaciones. Analizamos si, tal como se recoge en la bibliografía, la preferencia por rostros de hombres masculinizados se ve afectada por i) la etapa del ciclo menstrual, ii) el tener pareja estable y iii) el empleo anticonceptivos hormonales. No hemos podido reproducir el efecto de los dos primeros factores, pero sí encontramos que las mujeres que emplean anticonceptivos hormonales prefirieron rostros de hombre menos masculinos. Estos resultados no refutan la hipótesis del estro en humanos, pero nos indican que algunas de las pruebas que la sustentan han de ser reconsideradas, incorporando datos de poblaciones étnica y socioculturalmente diferentes.

Palabras clave: Estro; ciclo menstrual; dimorfismo sexual facial; valoración del atractivo; estrategias reproductivas.

Abstract: A recent and controversial hypothesis suggests the presence of an oestrus phase in women as in other mammals. This implies that women at their optimal fertility point of the menstrual cycle exhibit behaviors focused to maximize the genetic quality of their offspring. Several studies support this hypothesis, finding that women in the fertile phase tend to prefer men with traits associated to phenotypic quality, such as greater facial masculinization and symmetry. We experimentally tested some of the observations supporting this hypothesis in a population of 810 young Spanish women. We analyzed whether the preference for masculinized male faces is affected by i) the phase of the menstrual cycle, ii) having a stable partner and iii) the use of birth control pills. We could not reproduce the effect of the first two factors, but we found that women using hormonal contraceptives tend to prefer men with less masculine faces. These results indicate that some of the evidences supporting the oestrus hypothesis in humans must be reviewed, incorporating data from different socio-cultural and ethnic populations.

Key words: Oestrus; menstrual cycle; facial sexual dimorphism; attractiveness; reproductive strategies.

Introduction

Evolutionary biology suggests that every living being tries to maximize its fitness, the individual's genetic contribution to future generations (Freeman and Herron, 2002). In organisms with sexual reproduction, the contribution of a given individual to the next generation does not exclusively rely on its ability to have viable and fertile offspring, but also on the ability of its mate (Darwin 1871). For this reason, election of the reproductive mate strongly affects individual's biological fitness. Therefore, it is expected for the mechanisms that lead decisions that affect reproductive behavior to be under strong selective pressure (Penke, Todd, Lenton and Fasolo, 2007). Such selective pressure has produced selective discrimination abilities to evaluate opposite-sex individuals as potential mates in all plants and animals that use sexual reproduction. Human beings are not exempt of this circumstance. In humans, such discriminative abilities are mainly psychological adaptations that increase reproductive success (Buss, Cosmides, Tooby and Barkow, 1992).

Physical attractiveness plays a major role in mate choice in humans (e.g. Bailey, Duranteb and Geary, 2011; Buss, 1989; Rhodes, 2006; Rhodes, Chan, Zebrowitz and Simmons, 2003; Rhodes, Simmons and Peters, 2005). From an evolutionary perspective, perception of attractiveness is an adaptation that leads to differential mating success in our species (e.g. Buss and Schmitt, 1993; Gangestad and Simpson, 2000; Hönekopp, Rudolph, Beier, Libert and Müller, 2007). As a consequence, those individuals harboring features that are attractive for the opposite-sex show a higher reproductive success. Evolutionary Psychology proposes that sexual preference towards individuals with particular characteristics is an adaptation which goal is finding appropriate mates (Fink and Penton-Voak, 2002; Thornhill and Gangestad, 1999). So, these attractive characteristics indicate a reproductive advantage in the individual that displays them. These attractive characteristics usually present a strong sexual dimorphism. In men sexual dimorphic characteristics are linked to a higher reproductive capacity by the effect of androgen levels on male physiology (Evans, 2004; Neu, Rauch, Rittweger, Manz and Schoenau, 2002). High androgen levels cause masculinization in secondary sexual characters. On the other hand, androgens are known to reduce immunocompetence of males (Folstad and Karter, 1992). Therefore, only a male with a good genetic condition can carry the immunological disadvantages that denote those markers developed under high androgen levels (the so called Handicap hypothesis, see Folstad and Karter, 1992). Hence, such characteristics are honest markers of the individual's high phenotypic quality, making them attractive for the opposite-sex (Little and Hancock, 2002; Little, Jones, Penton-Voak, Burt and Perrett, 2002; Neave, Laing, Fink and Manning, 2003).

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Recent research suggests that there is an oestrus in women (Gangestad and Thornhill, 2008; Thornhill, 2007) in opposition with previous considerations (reviewed in Thornhill, 2007). Oestrus would occur in women at the optimum point of fertility of the menstrual cycle, involving an attempt to maximize the genetic quality of their offspring throughout different physiological and behavioral mechanisms (Alvergne and Lummaa, 2009). This phenomenon could be integrated in a mixed reproductive strategy in women. During oestrus women would try to copulate with men showing traits that reveal a higher genetic quality, such as facial masculinity or symmetry (Gangestad and Thornhill, 2008; Penton–Voak and Perrett, 2000; Thornhill, 2007). On the contrary, women would seek to establish long term bonds with individuals displaying pro-social behaviors such as fidelity or a higher parental investment, related to lower masculinization levels (Little, Cohen, Jones and Belsky, 2007). Hence, mixed reproductive strategy would occur as follows: women would try to copulate with more masculine individuals during oestrus ensuring for her offspring a good genetic quality, whereas during the non-fertile phase of the menstrual cycle would pursue to copulate with less masculine individuals ensuring a higher parental investment for her offspring (Gangestad and Thornhill, 2008; Penton–Voak and Perrett, 2000; Thornhill, 2007). The strategy of copulating during the non-fertile phase of the cycle is known as "extended sexuality", and it is frequent among Old World Primates and other species with a high parental investment (Thornhill, 2007). The mixed reproductive strategy would explain why the optimum of fertility in the human female does not involve any particular external change to request males' attention as happens in other animal species in which oestrus is evident (e.g. Dunbar and Dunbar, 1974; Rowell, 1969). Also, the mixed strategy would explicate why during the fertile phase in the menstrual cycle women are more receptive to courtship (Guéguen, 2009a, 2009b) and infidelity is more frequent (Bellis and Baker, 1990).

Several studies show that women in the fertile phase of the menstrual cycle prefer men showing masculine traits, supporting the oestrus hypothesis. Preferences during oestrus are documented for masculinized faces (Johnston, Hagel, Franklin, Fink, and Grammer, 2001; Penton-Voak et al., 1999; Penton–Voak and Perrett, 2000; Perrett et al., 1998; Jones et al., 2005, 2008; Little, Jones, and DeBruine, 2008; Vaughn, Bradley, Byrd-Craven, and Kennison, 2010), for manly low-pitch voices (Feinberg et al., 2006; Puts, 2005), for muscled and dominant men (Gangestad, Simpson, Cousins, Garver-Apgar and Christensen, 2004; Gangestad, Garver-Apgar, Simpson, and Cousins, 2007) and for the body scent of men classified as dominant and masculine (Havlicek, Roberts and Flegr, 2005). In many of these studies, it has been observed that the use on hormonal contraceptives (e. g. the contraceptive pill) significantly affects oestrus suppressing its expression (e. g. Alvergne and Lummaa, 2009; Penton-Voak et al, 1999; Little et al., 2002; Jones et al., 2008). Also, it has been stated that the preference of fertile women for more masculine traits is more intense in individuals on a stable relationship (Havlicek et al., 2005; Little et al., 2002; Scheib, 2001). These observations help to sustain both the presence of an oestrus and the existence of a mixed reproductive strategy in women.

Nevertheless, some recent studies have found evidence contrary to the oestrus hypothesis (Harris, 2011; Peters, Simmons and Rhodes, 2009). In these studies it was not possible to replicate the preference for more masculine faces during the fertile phase of the menstrual cycle. These results are worth to be considered as they are sustained on very rigorous and precise experimental design. On their study, Peters and collaborators (2009) directly measured the participants' levels of ovarian hormones, being this approach more precise than standard method (such as Johnston et al., 2001; Penton–Voak and Perrett, 2000) where the value is estimated counting days from the last menstruation. Also, Harris (2011) replicates the methodology of the first paper in the field (Penton-Voak et al., 1999) rising the number of participants to 853 women. In both studies the oestrus hypothesis is criticized, as well as the generalization to the outside world of results obtained in a controlled lab situation. These criticisms have been replied for the hypothesis supporters arguing that i) the great number of different studies sustain the observations with a huge number of observations, ii) the use of unmodified faces introduces too many uncontrolled variables in the evaluation, and iii) oestrus hypothesis is not only supported by the preference of more masculine faces during the fertile phase but also for many other evidences (DeBruine et al., 2010).

The main objective of the present study is to confirm if, according to the oestrus hypothesis, the preference for masculine faces changes through the menstrual cycle. As we have exposed there is a need to replicate the studies since for some authors has been impossible to repeat these results (Harris, 2011; Peters et al., 2009). Our study was performed in a population of young adult women in Spain. Oestrus hypothesis had not been tested in a Spaniard population before. Also, we simultaneously tested in our population the described effects of hormonal contraceptives and of a stable relationship on this preference. According to literature, we expected to find a preference for more masculine faces during the fertile phase in women, and for less masculine faces the rest of the cycle. We also expected to reproduce that women using hormonal contraceptives tend to prefer more feminine men faces. Finally, and according to the oestrus hypothesis as a part of a mixed reproductive strategy, we hoped to find that women in a stable relationship tend to prefer more masculine faces. Particularly, such a preference should be more evident in women on the fertile phase of the cycle and on a stable relationship.

Method

Participants

A homogeneous population of female Spanish university students (N = 810) was employed in the current study. Aged between 18 and 29 years old ($M \pm SD$= 20.86 ± 2.10), all participants are included in the standardized age-group *youth* (see Esnaola, 2008). Study was performed along years 2005-2009. Participants were recruited as volunteers and contributed anonymously. We allowed to join the experiment participants showing potential setbacks for the study (e. g. hormonal therapy, anti-epileptic medication) although their data were subsequently discarded.

Construction of stimuli

Nine couples of men faces were generated to be used as stimuli in the study. Each couple is composed of a slightly-feminine and a slightly-masculine version of the same male face. Those modified faces were built after modifying average young male faces (aged 19 to 25) as described below. The sample of young male faces employed was obtained in previous studies (Sanchez-Pages and Turiegano, 2010). Images were taken in JPEG format with resolution 3264×2448 and captured at 3m under standard light conditions. Subjects were asked to pose with a neutral expression and to remove any facial adornment. Individuals signed their explicit consent to use their images with exclusive scientific purposes. F. J. Rohlf's free and open-source software package TPS from the Morphometrics website (see http://life.bio.sunysb.edu/morph/) was employed to generate the stimuli.

To generate the stimuli we followed two steps: 1) obtaining the different shapes to which photographs are to be transformed, and 2) modifying a set of men's photographs into the chosen shape. This transformed images were melted and the resulting image used in the experiments. The two described steps require working on the face's shape, defined as the information harbored in an image after correcting for size, position and orientation modifications (Klingenberg and McIntyre, 1998). The shape of the face was delimited by a set of reference points (*landmarks*, LMs). These same LMs (Figure 1A) have been previously used in other studies (Fink et al. 2005; Sanchez-Pages and Turiegano, 2010; Windhager, Shaefer and Fink, 2011) since they represent face shape and are easily and unambiguously identified (Fink et al. 2005). These 57 LMs were placed on each of the 392 images (319 of men and 73 of women), employing TPSdig2 software.

Average men and women face shapes are required to generate a masculinized and feminized version of each face shape (Figure 1B). To this purpose we used on one side 73 women faces, and on the other nine groups of randomized men photographs (some 30 images on each group). Average faces were generated employing men or women photographs and their mirror-images in order to obtain completely symmetrical face shapes, to avoid interferences caused by changes in perception of symmetry along the menstrual cycle (Oinonen and Mazmanian, 2007). TPSrelw software provided an average feminine shape, as well as nine average masculine faces from the average face shapes of the 30 male individuals from each group. The software extracts the information concerning the shape of the object (the face) erasing variation caused by position, orientation or scale, hence rendering an "average shape".

Afterwards, we proceeded to estimate the differential vector between homologous LMs for the nine masculine average faces compared to the average feminine face. Subsequently each LM in the masculine face holds a vector that transforms the masculine face into the average feminine face. To produce the feminized men faces, each shape was changed from the average masculine to the average feminine shifting the transformation vector halfway. To produce the masculinized faces, each average masculine face was separated from the average feminine face subtracting to each of the nine average photographs half the transformation vector.

Once nine masculinized face shapes and their corresponding nine feminized face shapes were generated, we produced the final images. We created the final face couples using TPSsuper software that allows deformation of images to a pre-defined shape. Faces to be transformed were chosen among a set of selected photographs from men with scarce facial hair. Using the same selection we created, also randomly, nine groups of 15 individuals that (together with their mirror image) were deformed into the masculinized and the feminized shape. Average of the 15 deformed images of these nine groups produced the 18 definitive photographs, since the same set of images was subsequently transformed to both the masculinized and feminized version of a predetermined average face. This gave rise to the two modified versions of the same face (Figures 1C and D).

Test performance

Study was performed in groups of 15-20 participants which identity remained anonymous. The complete set of 9 couples of men faces was shown to each participant (one masculinized and the other feminized). They were asked to choose the most attractive men on each couple, suggesting them not to think in a long term relationship. This forced election approach was employed because in competitive societies, such as western, people tend to exaggerate their answers when they face more than two choices (reviewed in Kawamura, 2011). The order of presentation of the couples as well as the position of the masculinized and feminized face was randomly changed between groups of participants to control the effect of relative position. Participants were required to quickly decide without considering in detail facial features. They were asked to individually write down their election and communication with other participants was not allowed during the study. Time was not restricted, but it

never took more than 15 minutes. After the nine rounds of choosing the most attractive face, participants filled up a short questionnaire regarding their stage of the menstrual cycle and some control questions.

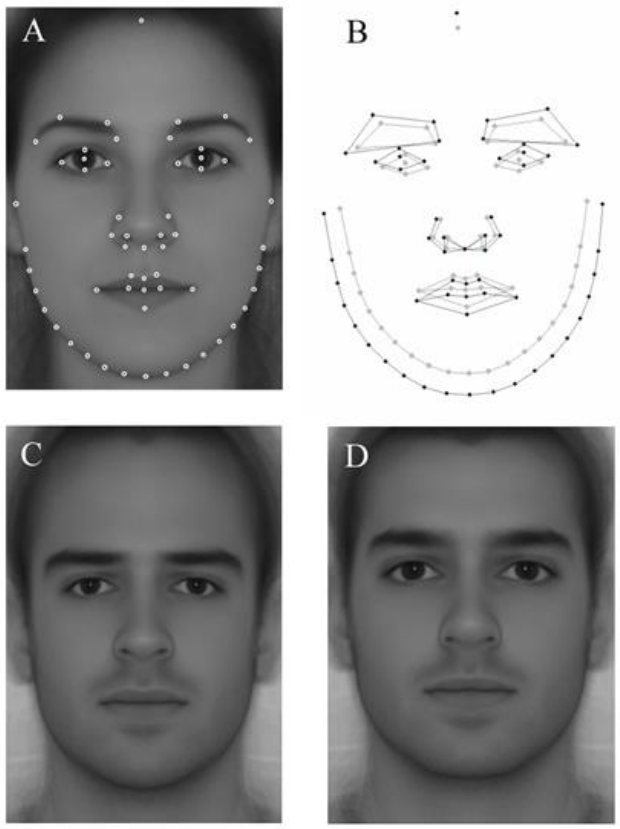

Figure 1. Generation of masculinized and feminized male faces. The 57 landmarks used to establish face shape are placed on the symmetrized average face obtained from 73 women faces (A). Average faces from men (black) and women (grey) (B). One couple of masculinized (C) and feminized (D) men faces used in the study.

Following other studies protocol (e. g. Penton–Voak and Perrett, 2000; Johnston et al., 2001), participants were asked to estimate their stage in the menstrual cycle based on their own timing (79.14% of participants affirmed having a regular cycle). They chose among three options: i) menstruation, ii) follicular phase y iii) luteal phase. Follicular phase should be considered if after menstruation they had not reached half the way of their regular standard cycle. If they were in the second half of their cycle after menstruation, they should consider themselves in the luteal phase. The final population was composed of 14.57% of women in menstrual phase, 36.42 % in follicular phase and a 49.01 % in luteal phase. These are consistent with randomly expected percentages, taking into account the duration of each stage in a 28 days cycle ($\chi^2_2 = .076$; $p = .783$). For further analysis, and according to literature (Penton-Voak et al., 1999; Penton-Voak and Perrett, 2000), we considered a high risk of conception to match with the follicular phase and a low risk with menstruation and luteal phase.

Also, participants were asked three questions related to variables that are known to interfere in the preference for masculine faces during the fertile period of the cycle. These questions were: 1) "Do you use hormonal contraceptives?" (Penton Voak et al., 1999; Little et al., 2002; Jones et al., 2008), 2) "Are you on a stable relationship?" (Havlicek et al., 2005; Little et al., 2002) and 3) "Are you pregnant?". We also asked if they were following any kind of drug treatment at that moment, in order to discard after the experimental session those employing drugs affecting behavior. They were also asked age and sexual orientation. This last question was asked to exclude homosexual individuals from the study, since we are unable to make predictions in that population about the changes in the attractiveness estimation of men just based on the oestrus hypothesis. Once excluded the homosexual population, 21.36% of the final sample used hormonal contraceptives, 49.88% was on a stable relationship and none was pregnant.

Statistical analysis

Participants significantly preferred the feminine face over the masculine five out of nine times (see Table 1). This is congruent with previously observed data in which women show a tendency to prefer feminized faces in the male population (although not in every case, as we observe). We performed a reliability test to confirm if our observations could reveal a tendency on each single participant to prefer a more masculine or more feminine face (Cronbach $\alpha = .688$).

Table 1. Preference for the masculine or the feminine face on each of the nine couples of photographs used as stimuli. Images appear in a preference for the more feminine order. In the percentage column bold characters show if the preferred option was the masculinized or the feminized face. Column of p-values shows in bold those results with statically significant differences in the preference of one of the faces.

	Transformation	*n*	%	*p*
Face 1	Feminized	662	**81.73**	**>.001**
	Masculinized	148	18.27	
Face 2	Feminized	651	**80.37**	**>.001**
	Masculinized	159	19.63	
Face 3	Feminized	607	**74.94**	**>.001**
	Masculinized	203	25.06	
Face 4	Feminized	575	**70.99**	**>.001**
	Masculinized	235	29.01	
Face 5	Feminized	470	**58.02**	**>.001**
	Masculinized	340	41.98	
Face 6	Feminized	412	**50.86**	.648
	Masculinized	398	49.14	
Face 7	Feminized	361	44.57	**.002**
	Masculinized	449	**55.43**	
Face 8	Feminized	267	32.96	**>.001**
	Masculinized	543	**67.04**	
Face 9	Feminized	223	27.53	**>.001**
	Masculinized	587	**72.47**	

As an index that represents the preference for a more masculine faces, we generated a variable adding the results

from each couple of faces. This variable "Preference for a masculinized face" (PMF henceforth) takes values from 9 (when the more masculine face is always preferred) to 0 (when the more feminine face is always chosen). Variable PMF showed a value of 3.780 ± .072 (*M* ± *SD*) which indicates a general tendency to prefer more feminine male faces, since the result is statistically significant different from the average value in the scale (t_{809} = -9.970; p <.001). Neither the variable nor its usual transformations display a normal distribution, hence, all statistical tests are non-parametric. In any case, they are all adequate analysis to study a variable presenting a low number of values (Conover, 1999). Analyses were performed employing SPSS15 software.

Results

In a first analysis, we considered the population not using hormonal contraceptives. We did not find significant differences in the PMF between women in fertile (n = 236) and non-fertile (n = 401) phase. This result is still consistent after discarding those participants who could have misestimated their stage in the menstrual cycle (participants with a non-regular menstrual cycle) (lower part of Table 2).

Table 2. Preference for a more masculine face in different hormonal stages. Results are shown for the complete population ("all" N = 810) and for participants with a regular menstrual cycle ("regular cycle" n = 652). The group "Non-fertile" does not include those participants using hormonal contraceptives.

	Comparison	*n*	*M ± SD*	*p*
ALL	Fertile	236	3.894±.132	.672
	Non-fertile	401	3.835±.103	
	Contraceptives	173	3.497±.154	.066
	Non-fertile	401	3.835±.103	
	Fertile	236	3.894±.132	**.041**
	Contraceptives	173	3.497±.154	
REGULAR CYCLE	Fertile	182	3.956±.154	.285
	Non-fertile	297	3.751±.119	
	Contraceptives	173	3.497±.154	.184
	Non-fertile	297	3.751±.119	
	Fertile	182	3.956±.154	**.032**
	Contraceptives	173	3.497±.154	

Table 2 also shows the comparison of the group of participants on the fertile stage with the control group, that is, those using hormonal contraceptives (n = 173). Women using hormonal contraceptives preferred more feminine men's faces. There are no significant differences in the PMF between participants in the non-fertile phase and the control group. Both results remain the same when we exclusively include in the analysis women with a regular menstrual cycle (see Table 2).

Women on a stable relationship did not show higher PMF. A preference for men with a more masculine face was not observed neither in the complete population nor when exclusively considering women in the fertile phase or women using hormonal contraceptives (Table 3).

Table 3. PMF in women with (n = 404) and without a stable relationship (n= 406), in fertile and non-fertile phase. Non-fertile group includes participants that use hormonal contraceptives.

Hormonal Stage	Stable Relationship		No Stable Relationship		*p*
	n	*M ± SD*	*n*	*M ± SD*	
Fertile	99	3.798±.204	137	3.964±.176	.497
Non-fertile	174	3.753±.164	227	3.899±.132	.426
Contraceptives	131	3.527±.177	42	3.405±.312	.878
Total	404	3.691±.104	406	3.869±.100	.163

Discussion

The purpose of our study was to perform in a Spanish population some of the experiments that sustain the oestrus hypothesis as a part of a mixed reproductive strategy in women. Specifically we studied the effect of three factors in women: i) their stage in the menstrual cycle, ii) having a stable relationship and iii) the use of hormonal contraceptives.

Our results in this group of 810 woman do not reproduce those obtained before (reviewed in Jones et al., 2008) in which the hormonal stage of participants (in a regular menstrual cycle) affected their preference for men faces. These studies observed that women in a high risk of conception are attracted by men with high masculinization in several features, like face shape. These results provided a strong support to the oestrus hypothesis in humans (Gangestad and Thornhill, 2008; Thornhill, 2007). We expected to replicate those results in our Spanish population. Nevertheless we did not find women in a fertile stage to prefer more masculinized men faces. That is, our results do not fit in the oestrus hypothesis. Results similar to ours have been previously found in other studies (Harris, 2011; Peters et al., 2009). Although controversial (DeBruine et al., 2010), these two studies present robust results in which changes were not found in face's preference along the menstrual cycle. Their robustness is provided by an accurate measurement of the hormone stage (Peters et al., 2009) and by the use of a higher number of participants than their preceding studies (Harris, 2011). Peters and collaborators (2009) measured levels of estrogens and progesterone in all their participants (n = 25). Hence, they used a more precise approach than most of the studies in the field (for example, Johnston et al., 2001; Penton–Voak and Perrett, 2000), in which hormone levels were estimated from the date of the last menstruation. Also, preference for more masculine faces was circumscribed to a short term relationship (Peters et al., 2009), additionally controlling if participants had a stable relationship. These two features had been described to positively affect the choice for more masculine faces during oestrus (e. g. Penton-Voak et al., 1999; Johnston et al., 2001; Little et al., 2002; Scheib, 2001). Lastly, and opposed to all previous studies, Peters and collaborators (2009) used real men faces as stimulus instead of allowing participants to modify the degree of masculinity of previously generated faces. Although this precise difference in the stimuli might explain their different results, authors criticized the oestrus hypothesis, especially concerned in the generalization

of results obtained in a particular experimental design. There is a second study in which preceding results could not be replicated (Harris, 2011). In this case, the methodology employed was the same as in the paper where the fluctuation in preference for male faces along menstrual cycle was first described (Penton-Voak et al., 1999). In fact, stimuli were the same in both studies. The strongest difference between these two studies relies in the number of participants (82 in Penton-Voak et al., 1999 against 853 in Harris, 2011). Moreover, Harris (2011) used a more precise method to estimate the stage of the menstrual cycle in participants (assessing the duration of the specific cycle in which participants took part in the study). In that study oestrus hypothesis is strongly criticized, suggesting related and supporting literature to be reconsidered (Harris, 2011).

These challenging results are enough evidence to perform additional studies to test the oestrus hypothesis. Their final purpose must be to clarify if that hypothesis should be reconsidered as a universal mechanism affecting human behavior. To achieve that goal it is necessary to replicate the results outside the Anglo-Saxon frame, in which the vast majority of studies have been performed. In a first approach the preference for masculinized faces was simultaneously analyzed in two different ethnic and sociocultural populations (Japanese and British, in Penton-Voak et al., 1999). Subsequent studies have predominantly been performed in British university students. Analyzing different human populations allows determining if factors affecting behavior are truly universal, with a physiological base and independent of ethnic or sociocultural bases. Hence, studies in different sociocultural populations are required to assess the effect of biological factors determining any human behavior. Particularly if we bear in mind that the effect of hormone administrations on behavior is highly variable accounting on the participant's idiosyncrasy (Eisenegger, Naef, Snozzi, Heinrichs and Fehr, 2010).

As mentioned above, results were also challenging when we analyzed only women with a regular cycle. We initially included in the analysis women with a non-regular cycle because a 3-4 day variation in the cycle (which is average variation in women with a non-regular cycle) should not affect the estimation of the stage. Our results equally failed to fit the initial hypothesis when we discarded participants with non-regular menstrual cycle (see Table 2). This leads us to openly criticize the approach to estimate the stage of the menstrual cycle. Even when we have followed strictly the procedure, we cannot deny that it is quite imprecise. Nevertheless, despite its imprecision, it is the most commonly used method in the literature (e. g. Penton-Voak et al., 1999; Penton–Voak and Perrett, 2000; Johnston et al., 2001). Its imprecision is evidenced by the fact that the authors subtly modify their approach in different studies (Penton-Voak et al., 1999; Penton-Voak and Perrett, 2000). Consequently, it could be argued that our impossibility to reproduce previous results supporting the oestrus hypothesis could be caused by an imprecise estimation of the cycle stage. Nevertheless, following precisely the same estimation method used in other studies that revealed an influence of the menstrual cycle stage in face preference should have rendered us similar results to those. That is not the case, and then the menstrual stage estimation does not utterly explain the difference between our results and previous studies. In fact, the estimation approach, although imprecise, might be useful enough to allocate in the fertile stage most of the fertile women in a population. Our inability to replicate previous studies can be alternatively explained by other causes. It could occur that the association between menstrual stage and face preference is not universal or depends on other factors not considered in the current literature so far. As we pointed out studies in which hormone levels were precisely measured did not replicate expected results either (Peters et al., 2009).

Additionally, the effect of being involved in a stable relationship on face preference was not replicated either in our study. As other previous studies we tried to replicate in a Spanish population the situation in which those women on a stable relationship tend to prefer more masculine faces (Little et al., 2002; Scheib, 2001). More than supporting the existence of an oestrus in women, this situation sustains the existence of a mixed reproductive strategy (although both hypotheses are strongly related). Considering both hypotheses together, we expected to find that preference for more masculine faces in women on a stable relationship should be remarked during the fertile stage of the cycle. However, we could not reproduce those results. We cannot affirm that being involved in a stable relationship determines a preference for more masculine faces, not even in the case of individuals on the fertile stage of the menstrual cycle. These negative results can be explained if the mixed reproductive strategy is not a universal phenomenon. Alternatively there are other likely explanations based on the methodology employed to categorize participants as involved in a stable relationship. We employed a single question, following the method employed in the study that previously described the effect of being on a stable relationship in face preference (Little et al., 2002). However, it in unarguable that a single simple question provides scarce information since it does not inform if participants are involved in a long term relationship (which is decisive for a mixed reproductive strategy), or about the degree of satisfaction. This last aspect could have masked the results, since in certain cases women currently in a stable relationship could be looking for a stable relationship with a different man, not just for a reproductive couple. Therefore, according to the mixed reproductive strategy hypothesis, they would not reveal a preference for more masculine faces, but the contrary.

Finally, we have been able to replicate results proposing that women using hormonal contraceptives prefer more feminine male faces (compared to those in the fertile stage of the cycle and not using contraceptives). Our results confirm those studies in which women employing hormonal contraceptives reveal different male face preferences than those not using them (Gangestad and Thornhill, 2008; Jones et al.,

2008; Little et al., 2002). Hormonal feminine contraceptives (such as birth-control pills) regulate levels of both estrogen and progesterone reproducing the hormonal stage in pregnancy and avoiding ovulation, and hence impeding fertilization (Alvergne and Lummaa, 2009). In this scenario the use of contraceptives could be a factor eliminating the feminine oestrus, and, therefore, affecting reproductive-related behavior such as the lack of preference for features revealing a higher phenotypic quality, like more masculine faces (Alvergne and Lummaa, 2009). Beyond their scientific interest, these results also provide a reliable support for our methodology.

In this study we evaluated the oestrus hypothesis considering a single feature, facial masculinity. Oestrus hypothesis is supported by a robust set of experiments analyzing several morphological, psychological and behavior-related features (Alvergne and Lummaa, 2010; Gangestad and Thornhill, 2008; Thornhill, 2007). For this reason we believe our study does not tear down oestrus hypothesis, not even considering it together with other mentioned studies (Harris, 2011; Peters et al., 2009). However, our results allow us to suggest that the oestrus hypothesis must be tested in populations with different ethnical origins, cultures and social backgrounds other than just Anglo-Saxon university students. Other interesting features that are also known to be affected by the menstrual cycle stage, besides facial attractiveness, should be simultaneously analyzed, such as perception of symmetry or voice masculinity.

Conclusion

The main conclusion of this study is the further need of a wider analysis to confirm the preference of women for more masculine faces during the fertile stage of the menstrual cycle as a universal feature. We recommend it since we used a large Spanish population sample and were not able to replicate previously published results, and ours was not the first case of a negative result. Therefore, we do believe that a larger set of studies is required to analyze the universality of the phenomenon. Especially since most of the previous results were obtained exclusively with subjects of study from British universities. As far as we know, ours is the first study with a large number of participants in a population of a different sociocultural background.

Based in our results, we also conclude that the method to estimate fertility of participants should be more accurate, preferably employing direct hormone measurement, or at least estimations of the stage of the cycle based on precise measurement of its duration. The method used in previous studies, that we also followed, is too imprecise to reassure that the preference fluctuations have a hormonal basis. Also, it should be reconsidered to what extent having a stable couple affect face preferences. Particularly it should be improved the shallow method employed to obtain that information from the experimental subjects. Having a stable couple involves several social and cognitive emotional processes that should be deeply considered to analyze its importance as a variable in this environment. Necessity of reconsidering both methods and results does not exclusively arise from our inability to replicate results; it has been claimed in two previous studies (Harris, 2011; Peters et al., 2009). One of them involves a quite large population and both employed precise mechanisms (or at least more precise than current) to assess the hormonal stage.

Finally we want to remark that our results suggest that some unconsidered factors could sharpen up the effect of physiology also in features with a strong biological backgrounds, particularly social and cultural factors that are present in a whole community (not different among any individuals of the same human group). It is important to comprehend to what extent physiology affects a certain behavior, but also to what extent that effect can be tempered by other factors.

Limitations

Estimation of the fertile stage, as well as being in a stable relationship, might have not been precisely enough estimated, despite using the same protocol as in previous studies (Penton-Voak et al., 1999; Penton-Voak and Perrett, 2000; Perrett et al., 1998; Jones et al., 2005, 2008; Little et al., 2008). Regarding estimation of the fertile stage, not being able to collect direct hormonal data, the estimation after the conclusion of the menstrual cycle could have been more accurate. Regarding the considerations of participants as involved in a stable relationship, it should be also considered the degree of implication of the individuals on their relationship as well as their satisfaction with their current couple, and, if possible, if that is a relationship with a reproductive objective.

On the other hand, although we guide the participants to evaluate faces from a short term relationship perspective, that was not openly remarked (to avoid the explanation to cause moral debate that could affect the results of investigation). However, the lack of precise explanation could have caused some participants to evaluate male faces as a long term couple or an extra-couple relationship (Penton-Voak et al., 1999), missing a short term consideration in their evaluation.

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References

Alvergne, A., and Lummaa, V. (2009). Does the contraceptive pill alter mate choice in humans? *Trends in Ecology & Evolution, 25*, 171-179.

Bailey, D. H., Duranteb, K. M., and Geary, D. C. (2011). Men's perception of women's attractiveness is calibrated to relative mate value and dominance of the women's partner. *Evolution and Human Behavior, 32*, 138-143.

Bellis, M. A., and Baker, R. R. (1990). Do females promote sperm competition? Data for humans. *Animal Behaviour, 40*, 997-999.

Buss, D. M. (1989). Sex differences in human mate preferences: Evolutionary hypotheses tested in 37 cultures. *Behavioral and Brain Sciences, 12*, 1-49.

Buss, D. M., Cosmides, L., Tooby, J., and Barkow, J. H. (1992). Mate preferences mechanisms: Consequences for partner choice and intrasexual competition. In *The adapted mind: Evolutionary psychology and the generation of culture* (pp. 249-266). New York: Oxford University Press.

Buss, D. M., and Schmitt, D. P. (1993). Sexual strategies theory: An evolutionary perspective on human mating. *Psychological Review, 100*, 204-232.

Conover, W. J. (1999). *Nonparametric statistics* (3 ed.). New York: John Willey & Sons

Darwin, C. (1871). *The descent of man, and selection in relation to sex*. London: Murray.

DeBruine, L. M., Jones, B. C., Frederick, D. A., Haselton, M. G., Penton-Voak, I. S. & Perrett, D. I. (2010). Evidence for menstrual cycle shifts in women's preferences for masculinity: A response to Harris (in press) "Menstrual cycle and facial preferences reconsidered". *Evolutionary Psychology, 8*, 768-775.

Dunbar, R. I. M., and Dunbar, P. (1974). The reproductive cycle of the gelada baboon. *Animal Behaviour, 22*, 203-204.

Eisenegger, C., Naef, M., Snozzi, R., Heinrichs, M. & Fehr, E. (2010). Prejudice and truth about the effect of testosterone on human bargaining behaviour. *Nature, 463*, 356-359

Esnaola, I. (2008). El autoconcepto físico durante el ciclo vital. *Anales de Psicología, 24*, 1-8.

Evans, N. A. (2004). Current concepts in anabolic-androgenic steroids. *American Journal of Sports Medicine, 32*, 534–542.

Feinberg, D. R., Jones, B. C., Law Smith, M. J., Moore, F. R., DeBruine, L. M., Cornwell, R. E., ... Perrett, D. I. (2006). Menstrual cycle, trait estrogen level, and masculinity preferences in the human voice. *Hormones and Behavior, 49*, 215-222.

Fink, B., Grammer, K., Mitteroecker, P., Gunz, P., Schaefer, K., Bookstein, F. L., ... Manning, J. T. (2005). Second to fourth digit ratio and face shape. *Proceedings of the Royal Society London, Series B, 272,* 1995-2001.

Fink, B., and Penton-Voak, I. S. (2002). Evolutionary psychology of facial attractiveness. *Current Directions in Psychological Science, 11*, 154-158.

Folstad, I., and Karter, A. J. (1992). Parasites, bright males and the immunocompetence handicap. *American Naturalist, 139*, 603-622.

Freeman, S., and Herron, J. C. (2002). *Análisis evolutivo* (2 ed.). Madrid: Pearson Educación.

Gangestad, S. W., Garver-Apgar, C. E., Simpson, J. A., and Cousins, A. J. (2007). Changes in women's mate preferences across the ovulatory cycle. *Journal of Personality and Social Psychology, 92*, 151-163.

Gangestad, S. W., and Simpson, J. A. (2000). The evolution of human mating: Trade-offs and strategic pluralism. *Behavioral and Brain Sciences, 23*, 573-644.

Gangestad, S. W., Simpson, J. A., Cousins, A. J., Garver-Apgar, C. E., and Christensen, P. N. (2004). Women's preferences for male behavioral displays change across the menstrual cycle. *Psychological Science, 15*, 203-207.

Gangestad, S. W., and Thornhill, R. (2008). Human oestrus. *Proceedings of the Royal Society London, Series B, 275*, 991-1000.

Guéguen, N. (2009a). Menstrual cycle phases and female receptivity to a courtship solicitation: An evaluation in a nightclub. *Evolution and Human Behavior, 30*, 351-355.

Guéguen, N. (2009b). The receptivity of women to courtship solicitation across the menstrual cycle: A field experiment. *Biological Psychology, 80*, 321-324.

Harris, C. R. (2011). Menstrual cycle and facial preferences reconsidered. *Sex Roles, 64*, 669-681.

Havlicek, J., Roberts, S. C., and Flegr, J. (2005). Women's preference for dominant male odour: Effects of menstrual cycle and relationship status. *Biology Letters, 1*, 256-259.

Hönekopp, J., Rudolph, U., Beier, L., Liebert, A., and Müller, C. (2007). Physical attractiveness of face and body as indicators of physical fitness in men. *Evolution and Human Behavior, 28*, 106-111.

Johnston, V. S., Hagel, R., Franklin, M., Fink, B., and Grammer, K. (2001). Male facial attractiveness: Evidence for a hormone-mediated adaptive design. *Evolution and Human Behavior, 22*, 251-267.

Jones, B. C., DeBruine, L. M., Perrett, D. I., Little, A. C., Feinberg, D. R., and Law Smith, M. J. (2008). Effects of Menstrual Cycle Phase on Face Preferences. *Archives of Sexual Behavior, 37*, 78-84.

Jones, B. C., Little, A. C., Boothroyd, L. G., DeBruine, L. M., Feinberg, D. R., Law Smith, M. J., ... Perrett, D. I. (2005). Commitment to relationships and preferences for femininity and apparent health in faces are strongest on days of the menstrual cycle when progesterone level is high. *Hormones and Behavior, 48*, 283-290.

Kawamura, K. (2011). A model of public consultation: Why is binary communication so common. *The Economic Journal, 121*, 819-842.

Klingenberg, C. P., and McIntyre, G. S. (1998). Geometric morphometrics of developmental instability: Analyzing patterns of fluctuating asymmetry with Procrustes methods. *Evolution, 52*, 1363-1375.

Little, A. C., Cohen, D. L., Jones, B. C., and Belsky, J. (2007). Human preferences for facial masculinity change with relationship type and environmental harshness. *Behavioral Ecology and Sociobiology, 61*, 967-973.

Little, A. C., and Hancock, P. J. (2002). The role of masculinity and distinctiveness in judgements of human male facial attractiveness. *British Journal of Psychology, 93*, 451-464.

Little, A. C., Jones, B. C., and DeBruine, L. M. (2008). Preferences for variation in masculinity in real male faces change across the menstrual cycle. *Personality and Individual Differences, 45*, 478-482.

Little, A. C., Jones, B. C., Penton-Voak, I. S., Burt, D. M., and Perrett, D. I. (2002). Partnership status and the temporal context of relationships influence human female preferences for sexual dimorphism in male face shape. *Proceedings of the Royal Society of London, Series B, 269*, 1095-1100.

Neave, N., Laing, S., Fink, B., and Manning, J. T. (2003). Second to fourth digit ratio, testosterone and perceived male dominance. *Proceedings of the Royal Society of London, Series B, 270*, 2167-2172.

Neu, C. M., Rauch, F., Rittweger, J., Manz, F., and Schoenau, E. (2002). Influence of puberty on muscle development at the forearm. *American Journal of Physiology - Endocrinology and Metabolism 283*, E103–E107.

Oinonen, K., and Mazmanian, D. (2007). Facial symmetry detection ability changes across the menstrual cycle. *Biological Psychology, 75*, 136-145.

Penke, L., Todd, P. M., Lenton, A. P., and Fasolo, B. (2007). How self-assessments can guide human mating decisions. In G. Geher and G. F. Miller (Eds.), *Mating Intelligence: New insights into intimate relationships, human sexuality, and the mind's reproductive system* (pp. 37-75). Mahwah: Lawrence Erlbaum.

Penton-Voak, I. S., and Perrett, D. I. (2000). Female preference for male faces changes cyclically: Further evidence. *Evolution and Human Behavior, 21*, 39-48.

Penton-Voak, I. S., Perrett, D. I., Castles, D. I., Kobayashi, T., Burt, D.,, and Murray, L. K. (1999). Menstrual cycle alters face preference. *Nature, 399*, 741-742.

Perrett, D. I., Lee, K. J., Penton-Voak, I. S., Rowland, D., Yoshikawa, S., Burt, D. M., ... Akamatsu, S. (1998). Effects of sexual dimorphism on facial attractiveness. *Nature, 394*, 884-887.

Peters, M., Simmons, L. W., and Rhodes, G. (2009). Preferences across the menstrual cycle for masculinity and symmetry in photographs of male faces and bodies. *PloS One, 4*, e4138.

Puts, D. A. (2005). Mating context and menstrual phase affect women's preferences for male voice pitch. *Evolution and Human Behavior, 26*, 388-397.

Rhodes, G. (2006). The evolutionary psychology of facial beauty *Annual Review of Psychology, 57*, 199-226.

Rhodes, G., Chan, J., Zebrowitz, L. A., and Simmons, L. W. (2003). Does sexual dimorphism in human faces signal health? *Proceedings of the Royal Society London, Series B (supplement), 70*, 93-95.

Rhodes, G., Simmons, L. W., and Peters, M. (2005). Attractiveness and sexual behavior: Does attractiveness enhance mating success? *Evolution and Human Behavior, 26*, 186-201.

Rowell, T. E. (1969). Intra-sexual behaviour and female reproductive cycles of baboons (Papio anubis). *Animal Behaviour, 17*, 159-167.

Sanchez-Pages, S., and Turiegano, E. (2010). Testosterone, facial symmetry and cooperation in the prisoners' dilemma. *Physiology & Behavior, 99*, 355-361.

Scheib, J. E. (2001). Context-specific mate choice criteria: Women's trade-offs in the contexts of long-term and extra-pair mateships. *Personal Relationships, 8*, 371-389.

Thornhill, R. (2007). The evolution of women´s estrus, extended sexuality, and concealed ovulation, and their implications for human sexuality research. In S. W. Gangestad and J. A. Simpson (Eds.), *The Evolution of Mind. Fundamental Questions and Controversies*. New York: Guilford Press.

Thornhill, R., and Gangestad, S. W. (1999). Facial attractiveness. *Trends in Cognitive Science, 3*, 452-460.

Vaughn, J. E., Bradley, K. I., Byrd-Craven, J., and Kennison, S. M. (2010). The effect of mortality salience on women's judgments of male faces. *Evolutionary Psychology, 8*, 477-491.

Windhager, S., Shaefer, K., and Fink, B. (2011). Geometric morphometrics of male facial shape in relation to physical strength and perceived attractiveness, dominance, and masculinity. *American Journal of Human Biology, 23*, 805-814.

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