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„Sexual Swelling as Honest Signal for Fertility in Female Barbary Macaques (*Macaca sylvanus)*?

A 3D Measurement Study to Analyse the Association between Female Perineal Swelling, Hormonal Status and Male Interest“

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Abstract

Barbary macaques (*Macaca sylvanus*) are seasonal breeders living in multimale- multifemale groups. Females display conspicuous swellings of the anogenital region to signal fertility. Tumescence and detumescence are regulated by hormones associated to the ovarian cycle. Sexual swellings have a strong influence on intra- and intersexual interactions and advertise female attractiveness. However, whether perineal swellings signal female fertility reliably is still a topic of debate. In this study the morphology of this feature is analysed applying a 3D geometric, respectively, morphometric method. In 2D measurement studies length and width of sexual swellings calculated from pictures are used to determine swelling expressions. This results in a measurement of the swelling area but not of the volume. In the recent study behavioural, physiological, and morphological data were analysed and tested whether male interest in females coincides with maximal swelling tumescence and female ovulation (measured by estrogen to progesterone ratios(EP)), as well as with female's presentation behaviour. Based on the findings of previous studies male interest is expected to be increased at the ovulatory phase within the female cycle when sexual swellings are supposed to be maximally swollen in association with elevated EP. Data collection consisted of behavioural observation, of gathering urinary samples to determine sexual hormones, and video recordings of the anogenital region in a free- ranging population located at the Rock of Gibraltar. The results showed that males had increased interest in females in relation to elevated female presentation rates and maximal swelling volumes. No significant correlation between EP and male interest was shown. From these results it is concluded, that female presentation behaviour and the volume of sexual swellings may play an important role in signalling fertility, respectively, attractiveness and have therefore, a strong influence on male interest. The missing relation between EP and male interest differs to studies using a 2D approach. The latter might suggest a difference between 2D and 3D methods insofar, that the 3D approach signals a more specific information about female attractiveness, which is not necessarily related to ovulation.

Keywords: Barbary macaque, female sexual swelling, hormone status, reliable signal, male interest

Introduction

In most mammals, females are receptive only during the fertile phase of the ovarian cycle (Brauch et al., 2007). Receptivity is defined in terms of female responses necessary and sufficient for the male's success to achieve intravaginal ejaculation and fertilization (Beach, 1976). Thus the window for a possible fertilization is usually rather small, e.g., in rodents, ranging from a few hours to a couple of days depending on the species. To synchronize fertile periods and male sexual interest, females may indicate their fertility to males by visual and behavioural signals, but also for example by vocal and tactile cues (Young et al., 2013).

Such signals have evolved in diverse taxa (Pagel, 1994). For instance, some species express changes in coloration of the anogenital skin, e.g., vervets (*Cercopithecus aethiops*) and patas monkeys (*Erythrocebus patas:* Dixon, 1998). Rhesus macaques (*Macaca mulatta)* show an increased coloration of the face during the periovulatory phase (Dubuc et al., 2009). In female Japanese macaques (*Macaca fuscata*) coloration intensity of the hindquarters, faces, and nipples seems to be associated with sexual attractiveness (Wallner et al., 2011). Other females of several Old World primates show changes in tumescence of the external genitalia during the periovulatory period of the ovarian cycle (Dixson, 1983). Generally, large anogenital swellings are found in species in which reproduction is not restricted to a breeding season. Barbary macaques (*Macaca sylvanus)* are special in this respect as they exhibit exaggerated swellings of the circumanal region and the labia although they reproduce seasonally (Küster & Paul, 1984; Ménard et al., 1985; Taub, 1978). These macaques live in multimale - multifemale groups. Therefore, female-female competition for matings with high-quality males at the time of highest fertility may have triggered to display conspicuous swellings of the anogenital region to signal fertility (Heistermann et al., 2006; Higham et al., 2012).

Swelling size changes over the female cycle and tumescence and detumescence are related to sex steroids (Küster et al., 1984; Möhle et al., 2005;

Wallner et al., 2006). While estrogen provides a stimulatory effect, progesterone antagonizes this effect (Carlisle et al., 1981; Gordon et al., 1991). Therefore, swelling size increases in the course of the follicular phase of the ovarian cycle, reaches its maximum during the periovulatory period, and is followed by detumescence related to activity of the *corpus luteum* (Aujard et al., 1998; Wildt et al., 1977). As Möhle et al. (2005) describe, swelling cycles are characterized by progressive size increases from the early to the late follicular phase in association with an elevated estrogen to progesterone (EP) ratio and ovulation occurs during the maximum swelling phase. In contrast, a decrease in swelling size corresponds to a decrease in estrogen levels with a rise in progesterone concentrations, and thus results in a return of the EP ratio to low baseline levels. Furthermore, Barbary macaques exhibit sexual swellings at day 18- 30 of gestation also caused by elevated EP ratio (Möhle et al., 2005).

For males, especially those living in multimale-multifemale groups, it is important to perceive the optimum time for copulation and conception (Bereovitch, 1987), because such societies show promiscuitive mating behaviour in relation to intra-males competition. To what extent males can utilise sexual swellings as a source of information about female fertility varies between species (Young et al., 2013). Engelhardt et al. (2004) argue that there is little empirical evidence about male primates being able to detect females' fertile phases. Although changes in swelling size seem to provide some information about female reproductive status, variations within and between species exist regarding the length of maximum tumescence and the timing of ovulation within this period (Deschner et al., 2003; Engelhardt et al., 2005; Reichert et al., 2002). Küster and Paul (1984) argue that swelling size is not a reliable indicator of female cycle stage in Barbary macaques. Ovulation usually occurs immediately before sex skin detumescence, but this association is not perfect, and ovulation has repeatedly been documented at other times of the swelling cycle (Dixson, 1983; Martin, 1992; Small, 1993).

Primate sexual swellings are hormone-dependent signals and may have a strong influence on socio-sexual interactions between individuals of a group (Higham et al., 2008; Wallner & Dittami, 1997). For example mating behaviour and female attractiveness vary in the course of the ovarian cycle (Heistermann et al., 2008). Attractiveness refers to female's stimulus extent in evoking sexual responses by the male (Beach, 1976). Heistermann et al. (2008) found that copulation rates increase towards the time of ovulation with a distinct peak of ejaculatory (but not non-ejaculatory) copulations in the fertile phase. Males inspect females most frequently during the fertile phase. Ejaculatory copulations and male pericopulatory behaviours are correlated with levels of female sex hormones (Heistermann et al., 2008). Furthermore, ejaculatory copulations and inspection rates are increased as well in the pre-fertile period compared to the post-fertile period. No differences in frequencies are found between pre-fertile and fertile periods (Young et al., 2013). Thus, copulations occur not only exactly at the time of ovulation, but also around (before and after) the fertile period, when sexual swellings are not fully developed.

Also female sexual behaviour varies remarkably throughout the ovarian cycle (Brauch et al., 2007). Taub (1980) described that estrous Barbary macaque females take an active part in the formation of sexual associations (Taub, 1980). But, although females show cyclic changes in sexual behaviour and anogenital swellings it is largely unclear to what extent these sexual signals provide reliable information about the timing of the fertile phase (Brauch et al., 2007; Zinner et al., 2002). The significance of anogenital swellings as reliable indicators for ovulations, is still unresolved (Möhle et al., 2005) and the information content of these secondary sexual characteristics not yet fully understood (Sonnweber et al., 2013).

Nunn (1999) argues that the gradual increase of sexual swelling size may have important implications for the type of information that is conveyed. Nunn's (1999) *graded signal hypothesis* suggests that exaggerated swellings can be regarded as sexual signals indicating the probability of ovulation with swellings being largest around the time of highest fertility, and swellings being smallest when the probability of the female being fertile is lowest. Ovulation should occur close to peak swelling with decreasing probability of ovulation before and after maximum tumescence. Thus, prominent swellings can be modelled as probability

distributions, which reflect the likelihood of ovulation (Nunn, 1999). This hypothesis suggests that exaggerated swellings function simultaneously to confuse paternity and skew the probability of paternity towards highest quality males. This may resolve two problems females face in multimale - multifemale societies: On the one hand they have to produce high quality offspring, which is achieved by biasing paternity towards the dominant male (which monopolizes females during the time of probable fertility) to increase the probability of obtaining good genes for their offspring. On the other hand, they have to assert their offspring's survival by avoiding potential infanticide. Less dominant males may achieve copulations during periods of lower probability of fertility (Nunn, 1999). By mating with multiple males the risk of infanticide can be reduced because males are less likely to kill infants they might have sired (Hrdy, 1979; van Schaik et al., 1999).

In contrast, Hamilton (1984) proposed that exaggerated swellings pinpoint the timing of ovulation and thus increase paternity certainty. This *obvious- ovulation hypothesis* predicts that sexual swelling size honestly signals ovulation.

Both of these within-cycle hypotheses build on the notion that the timing of ovulation is correlated with the period of maximal swelling tumescence within each estrous cycle (Alberts & Fitzpatrick, 2012). However, sexual swellings might signal multiple types of information relevant to fertility (Alberts & Fitzpatrick, 2012; Fitzpatrick, 2014). The *reliable indicator hypothesis* proposes that in multimale - multifemale groups the size of sexual swelling honestly advertises female fitness and is therefore an indicator for female quality (Pagel, 1994). For example, wild baboon females with larger swellings reach sexual maturity earlier, and produce both, more offspring and more surviving offspring per year, than females with smaller swellings (Domb & Pagel, 2001).

Most published studies regarding the morphology and expression size of sexual swellings have been restricted to 2D analytical tools so far. However, sexual swellings represent 3D structures. Therefore, analyses and descriptions of these signals based on 2D measurements may miss important, meaningful informations, which are included in the 3D dimension. In this study the morphology of this secondary sex character is analysed applying a 3D geometric, respectively, morphometric method. The 3D-nature of soft-tissues can be quantified by computing their volume using landmarks found directly on the specimen of interest (Sonnweber et al., 2013).

This work investigates whether sexual swellings in Barbary macaques can be classified as honest signals of fertility. Furthermore, research on caged macaques showed that females have repeated cycles with exaggerated swelling expressions even without ovulation (Wallner & Dittami, 1997). In this study behavioural, physiological, and morphological data are analysed and tested whether male interest in females coincides with maximal swelling tumescence and female ovulation (EP ratios), as well as with female's presentation behaviour in relation to male interest.

Material and Methods

Subjects and study site

The study was carried out on a population of free-ranging Barbary macaques (*Macaca sylvanus)* living at the Rock of Gibraltar. Two groups were observed during the reproductive period from October 2005 to March 2006 (Sonnweber et al., 2013). The groups of "Apes Den" (AD) and "Prince Phillips Arch" (PPA) were chosen due to their good accessibility and due to their comparable group composition. AD consisted of 14 adult females, nine adult males, four subadult females, five subadult males, five juveniles and seven infants. The PPA group was composed of 12 adult females, nine adult males, six subadult females, six subadult males, eight juveniles and seven infants. Data were collected on six focal females per group, in total 12 individuals. They represented all age classes within the group of mature females. Only in this sub-sample sufficient hormonal, behavioural, and swelling data were provided from intact females. Hormone samples, video recording of sexual swellings, and focal behavioural protocols were collected for all focal individuals. All 12 females were cycling during the study, but some were nursing offspring from the previous mating season (Sonnweber et al., 2015). All data collection methods were of non-invasive character. Videos (filmed with a Sony Mini DV Digital Handycam) of female swellings and other data were recorded every second day alternating

Figure 1. Series of Pictures that were used for landmarking with PhotoModeler ® Scanner 2011 software. The pictures represent a general view of the caudal and caudo- lateral perspectives to both sides of the swelling. *The photographs are courtesy of Mag. Dr. Ruth S. Sonnweber.*

between groups (Sonnweber et al., 2013). Daily observations took place from 08:00 to 17:00 (Sonnweber et al., 2015).

The recent study focusses on data of five out of the 12 focus females mentioned above. The reason for this selection was that they coincide in particular characteristics. All of them conceived and delivered infants in June and July 2006. Average gestation length (170 days in macaques (Möhle et al., 2005)), were calculated back from the date of birth of the offspring, was used to estimate the timing of fertilization. All five focal females were fertilized during the time of observation, which indicates the presence of conceptional and non-conceptional swelling cycles in this study. The focal individuals were born between 1989 and 1999 (respectively the mean age was 10.6 ± 4 years at the time of observation). Two of the focal females had infants from the previous mating season.

Behavioural observations

Sexual interactions were recorded by using continuous focal animal and ad libitum sampling (after Altmann, 1974) (Sonnweber et al., 2015). Individual focal protocols lasted 15 minutes. Individuals were observed in pseudorandom order. No female was observed more than once on the same day, and morning, midday, and afternoon observations were balanced across the focal females. Behavioural observations were limited by bad weather conditions or inaccessibility of specific areas on certain days (Sonnweber et al., 2015). Male interest in females included the following parameters: examination of female anogenital region (inspection), climbing onto the female for eventually occurring further actions (mount), insertion of the penis into the vagina without copulatory movements (intromission) and copulations with ejaculations to happen (ejaculatory copulations). Furthermore female presentation behaviour (female presenting their anogenital region to a male) was observed (Brauch et al., 2007).

Urinary sample collection and hormone analysis

Urine samples were collected whenever possible after a female urinated between 08:00 and 17:00, but only those samples were collected, where a cross-contamination of urine with feces can be excluded. Urine was soaked up from rocks, leaves and concrete around the feeding sites using a cotton roll and were immediately stored at - 20°C in salivettes (Sarstedt, Germany) until analysis. A total of 118 samples were collected from the five females, which results in an average of 23.6 samples per individual.

Before analysing estrogen and progesterone by applying an enzyme-immuno-assay (EIA), both steroids were deconjugated with glucuronidase/sulphatase (Palme and Möstl, 1993). Furthermore, all hormone concentrations were adjusted for urine dilution by analysing creatinine concentration according to the standard Jaffe method (Slot, 1965). Previous studies reported estrone, estriol, and estradiol (Monfort et al., 1984; Shideler et al., 1993, 2001) and pregnanediol conjugates respectively (French et al., 2004; Wasser et al., 1994) to be dominantly excreted in the urine of macaques and baboons. Therefore hydrolysed urine samples were analysed for estrogen concentrations using an antibody against total estrogens (Palme and Möstl, 1993) and pregnanediol-3-glucuronide (PdG) (Shidler et al., 2001; Shidler et al., 1985; Heistermann et al., 1995). Total estrogens were proven to be an accurate measure for female macaque reproductive functions (Heistermann et al., 2001; Ostner and Heistermann, 2003). Estrogen and progesterone metabolites accurately reflect female ovarian function in the

Barbary macaque (Möhle et al., 2005). Intra-assay coefficient of variation (CV) was 7.96% and inter-assay CV was 14.84% for estrogen analyses, and 16.51% and 17.38% for progesterone analyses respectively.

Determination of swelling volumes

As mentioned above, the 3D-nature of soft-tissues can be quantified by computing their volume using landmarks found directly on the specimen of interest. Video sequences of females` sexual swellings were recorded from the beginning of December 2005 to the middle of March 2006 (recordings from February 2006 missing in data), using a Sony Mini DV Digital Handycam and taping caudal, respectively, caudo- lateral positions. Single photos were extracted from the videos using Windows Movie Maker © 2012 Microsoft Corporation (windows.microsoft.com) for further processing. Each swelling expression was analysed with the PhotoModeler ® Scanner 2011 software. Three to five pictures of caudal and caudo- lateral positions were cut out of the mentioned video sequences. It was important to choose a series of pictures that represented a wide-angle view of the caudal and caudo- lateral perspectives to both sides of the swelling, and to use only pictures of individuals standing or lying without moving (Figure 1). In total 225 pictures of sexual swellings were analysed. For flat swellings three pictures were sufficient, whereas for fully tumescent signals five pictures were analysed. For flat swellings a minimum of 50 landmarks were used, whereas for highly developed swellings a minimum of 70 landmarks were used. The reason for the increased number of landmarks was that on highly developed swellings the region on which landmarks could be set also increased. Swelling volume calculation is achieved by combining existing photogrammetric and morphometric tools. The PhotoModeler® Scanner 2011 software (www.photomodeler.com) is used for coordinate acquisition, the Morpheus et al. software (Slice, 1998) for the orientation of the specimens, and finally the MATLAB R2014b software (www.mathworks.com) for calculating swelling volume (Figure 2) (Sonnweber et al., 2013). For detailed information about the method see elsewhere (Sonnweber et al., 2013).

Statistical analysis

All statistics were computed using R x64 3.2.0 (http://www.r-project.org) software. The dependent variable was male interest in female sexual attractiveness, including inspections, mounts, intromissions and ejaculatory copulations. Independent variables were volume of the swelling (VOL), estrogen to progesterone ratio (EP) and female presentation (FP) as well as the interactions of EP: VOL, EP: FP, VOL: FP and EP: VOL: FP in my models. Furthermore, as random effect, the individual was included. The analysis included fitting several regressions using linear mixed-effects model fit by maximum likelihood (ML). Model selection was based on different criteria: corrected Akaike Information Criterion (AICc) for small sample size, weighted AICc (wAICc), and Bayesian Information Criterion (BIC) scores (Burnham and Anderson, 2002). When models differ only marginally in their AICc and BIC scores (<10) they are to be treated as equivalent (Burnham and Anderson, 2002), with models with fewer variables being preferred over models with many variables. Weighted scores indicate probabilities of how likely a given model is the best model to fit of the set of available models (Sonnweber et al., 2015). Since no scaling factor for the coordinate data were available, relative volume size was calculated.

Results

First, several regressions were fitted and these models were compared against each other to find the model that explained variation in male interest best (Table 1). It was started with the full model containing volume of the swelling (VOL), estrogen to progesterone ratio (EP), female presentation (FP) and the interactions of VOL x EP, VOL x FP, EP x FP, and VOL x EP x FP. Applying a step-wise reduction methods variables were dropped and the new models were re- run until AICc scores no longer decreased. Model 3 had the lowest AICc score (Table1), indicating that it explains male interest best. This model contained EP ratio, FP, and the interactions between EP x VOL, EP x FP and FP x VOL. Significance of variable interaction was then tested with Fisher's Analysis of variance (ANOVA).

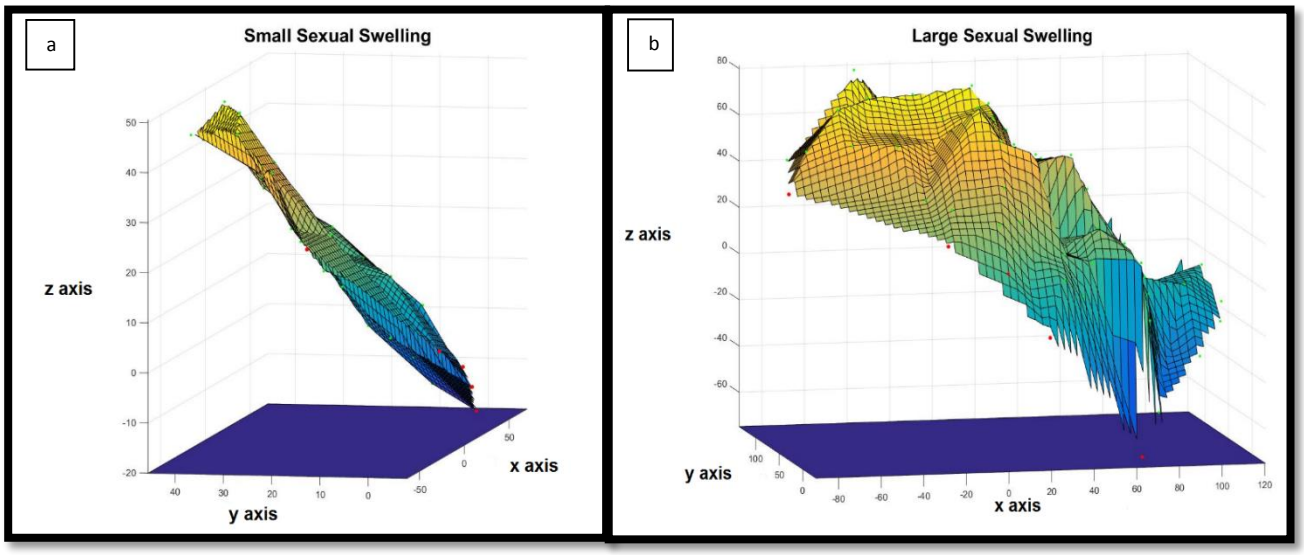

Figure 2. 3D- figures of sexual swellings of small (a) and large (b) volume after calculation with MATLAB R2014b software (www.mathworks.com). These figures show relative size of swelling since I did not have a scaling factor for the coordinate data.

In the final model (Table 2) female presentation (f= 105.30, p < 0.0001, df= 21)) was a significant predictor of male interest as well as the interaction between FP x VOL (f= 16.44, p= 0.0006, df= 21). Other fixed effects of this model, EP (f= 3.16, p=0.0897, df= 21), EP x VOL (f= 1.30, p= 0.2673, df= 21) and EP x FP (f= 3.29, p= 0.0842, df= 21) were not significant. Male interest in females is high when female presentation rates are high and also when the interaction between presentation and sexual swelling is high. The exact correlation between the interaction variables couldn't be defined due to the small dataset.

Table 1. Model comparison for dependent variable male interest

model	Independent variables	Independent variable interactions	loglikelihood	AICc	BIC	wAICc
1	VOL EP FP	VOL x EP VOL x FP EP x FP VOL x EP x FP	-47.52078	126.6205	129.0535	0.01
2	EP FP	VOL x EP VOL x FP EP x FP VOL x EP x FP	-47.55379	122.1076	125.7183	0.11
3	EP FP	VOL x EP VOL x FP EP x FP	-47.59075	118.0386	122.3911	0.85
4	FP	VOL x EP VOL x FP EP x FP	-52.88063	124.8522	129.5696	0.03

All models for explaining the dependent variable male interest included female presentation (FP), as well as the interactions between swelling volume (VOL) x estrogen to progesterone ratio (EP), VOL x FP and EP x FP. Model selection was based on AICc and BIC scores, as well as on wAICc values. All models differed only marginally with less than 10 units from each other in their AICc and BIC scores. For model 3 AICc value was lowest (AICc= 118.0386). wAIC score also indicated that model 3 was the best model to fit of the set of available models. AICc= corrected Akaike Information Criterion, BIC= Bayesian Information Criterion and wAICc = weighted corrected Akaike Information Criterion.

Table 2. Final Model

Independent variables	DF	*f- value*	*p- value*
EP	21	3.16	0.0897
FP	21	105.30	<0.0001
EP x VOL	21	1.30	0.2673
EP x FP	21	3.29	0.0842
FP x VOL	21	16.44	0.0006

The model that explained male interest best contained estrogen to progesterone ratio (EP), female presentation behaviour (FP) and the interactions between EP x swelling volume (VOL), EP x FP and FP x VOL. Female presentation ($p< 0.0001$) and the interaction of FP x VOL ($p= 0.0006$) were the significant predictors of male interest. DF = degrees of freedom.

Discussion

The results of this study show that sexual interest of male Barbary macaques varies predictably with female presentation behaviour and sexual swelling volume. Male interest in females is increased when female presentation rates are high and volumes of sexual swellings are large. No significant connection between EP ratios and male interest was detectable, but increased male interest tends to be associated with highest EP ratios.

As expected, sexual swelling expression is connected to male interest in females. This coincides with findings by Brauch et al. (2007) who demonstrated that frequencies of male ejaculatory copulations show a strong positive correlation with swelling size. It also supports the notion that copulations of males are more frequent on days of maximum swelling, irrespective of whether females were fertile or even already pregnant (Young et al., 2013). Therefore, swelling volume seems to be an important attractant for males. While Brauch et al. (2007) and Young et al. (2013) looked at the connection between 2D swelling size and ejaculatory copulations only, the current study examined variation in swelling volumes and general male interest (including ejaculatory copulations) in females. Despite the different methods applied the results of both studies hint in the same direction: female sexual swellings seem to be an important visual cue that affects male sexual interest. For future studies it will be interesting to look at male behaviour in more detail, for instance by analysing the correlation of 3D swelling volumes and ejaculatory copulations. A more fine-grained analysis of behavioural parameters in the future may allow for detecting subtle associations between changes in swelling volumes and variation in behaviour.

Furthermore, the results of this study indicate that male interest is increased when female presentation rates are high. Thus, besides sexual swelling volume female presentation behaviour seems to be an important factor in evoking male sexual interest. In mammals oestrus females often indicate their receptivity by behavioural actions towards the males and take part in establishing and maintaining sexual contacts (Beach, 1976). The Barbary macaques' mating system is primarily shaped by "female choice" (but see Küster & Paul, 1994) and females actively form and maintain sexual associations with males (Taub, 1980). This study's results demonstrate that males show

highest interest in females when females themselves show sexual interest. This is in line with findings by Young et al. (2013) who describe that male mating frequency is increased when female socio- sexual behaviour rates, namely female presentations, are high. Furthermore, in conception cycles males' interest is concentrated around the fertile phase, while this is not the case for post-conception cycles. This indicates that males infer information about the fertile phase from more than sexual swelling size (Young et al., 2013). Alternatively there may be variation in the expression of sexual swellings between conception and non-conception cycles. Further studies are needed to answer this question conclusively.

Signalling fertility seems to be a very complex matter, consisting of different components. This study and other findings demonstrate that it is not sexual swelling expression alone that may influence male interest: the interaction of different cues and signals may alter male interest in females. Female behaviour, morphological traits, and olfactory cues are potential signals indicating female fertile phases or female quality (Brauch et al., 2007). Hence the combination of these signals may be important when looking at variability in male interest.

Heistermann et al. (2008) point out that male Barbary macaques concentrate their reproductive effort (measured by rates of inspection of females' anogenital region, time spend in consortship and ejaculation rates) to the fertile phase. In this study the connection between male interest and EP ratio as well as of male interest and the interaction of EP x FP was only marginally significant. This is likely to be due to the low sample size. The trend of this study's data suggests that male interest and EP ratio are associated, which would also be in line with previous studies on Barbary macaques (e.g., Young et al., 2013). Both, males and females show highest rates of sexual behaviours around the time of ovulation (Young et al., 2013). While frequencies of inspection and ejaculatory copulations increase during ovulation. Other behaviours like mounts and non-ejaculatory copulations occur at similar rates throughout the female cycle (Heistermann et al., 2008). Therefore, it would be interesting to split this study's dependent variable, male interest, into single behaviours (inspection, mount, intromission and ejaculatory copulations).

The interaction of EP and swelling volume does not seem to influence male interest. Previous studies however suggest that swelling size correlates with female fertile periods (Wallner et al., 2007; Möhle et al., 2005) and that male sexual activity is highest around the time point of ovulation. Important characteristics of these 3D soft tissues might have been missed in previous studies applying 2D measurement tools. The 3D measurement method that allows calculating swelling volumes provides a more accurate quantification of sexual swellings than measuring swelling size using height and width only. As there might be more subtle variations in swelling size than assumed so far, 2D measurements may not suffice to capture changes in swelling expression. Möhle et al. (2005) measured swelling depth in addition to height and width from single 2D pictures. As accurately measuring depth proved to be difficult, they might still have missed some important information contained in swelling volume changes. This may explain the different results from the current study. Therefore, even very subtle changes in swelling volumes can be detected. The latter might suggest a difference between 2D and 3D methods insofar, that the 3D approach signals a more specific information about female attractiveness, which is not necessarily related to ovulation. Further research is needed to draw conclusions: it would be helpful to compare 2D versus 3D analysis by using the same data set to detect potential differences between these methods. In the recent 3D analysis relative swelling volumes were calculated instead of absolute volumes. For further research it would be interesting to examine absolute swelling volumes. Kuester and Paul (1996) describe that male mating activity is restricted to periods of increased levels of estrogen. Therefore, in addition to EP ratios, it would be of interest to have a detailed look at estrogen and progesterone changes in relation to swelling volumes.

The present study seems to be the first, that reveals results between the relationship of sexual swellings volumes, female sexual hormones and male interest. However, whether these 3D measured sexual traits signal fertility cannot be determined from this study. Further studies with

detailed hormonal profiles of female ovarian cycles, fine-grained analysis of male and female sexual behaviour, and swelling volume changes will help to accept or reject either of the two core hypotheses about these conspicuous sexual traits, the *graded signal hypothesis* and the *obvious ovulation hypothesis*.

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Anhang

Abstract

Barbary macaques (Macaca sylvanus) are seasonal breeders living in multimale- multifemale groups. Females display conspicuous swellings of the anogenital region to signal fertility. Tumescence and detumescence are regulated by hormones associated to the ovarian cycle. Sexual swellings have a strong influence on intra- and intersexual interactions and advertise female attractiveness. However, whether perineal swellings signal female fertility reliably is still a topic of debate. In this study the morphology of this feature is analysed applying a 3D geometric, respectively, morphometric method. In 2D measurement studies length and width of sexual swellings calculated from pictures are used to determine swelling expressions. This results in a measurement of the swelling area but not of the volume. In the recent study behavioural, physiological, and morphological data were analysed and tested whether male interest in females coincides with maximal swelling tumescence and female ovulation (measured by estrogen to progesterone ratios(EP)), as well as with female's presentation behaviour. Based on the findings of previous studies male interest is expected to be increased at the ovulatory phase within the female cycle when sexual swellings are supposed to be maximally swollen in association with elevated EP. Data collection consisted of behavioural observation, of gathering urinary samples to determine sexual hormones, and video recordings of the anogenital region in a free- ranging population located at the Rock of Gibraltar. The results showed that males had increased interest in females in relation to elevated female presentation rates and maximal swelling volumes. No significant correlation between EP and male interest was shown. From these results it is concluded, that female presentation behaviour and the volume of sexual swellings may play an important role in signalling fertility, respectively, attractiveness and have therefore, a strong influence on male interest. The missing relation between EP and male interest differs to studies using a 2D approach. The latter might suggest a difference between 2D and 3D methods insofar, that the 3D approach signals a more specific information about female attractiveness, which is not necessarily related to ovulation.

Zusammenfassung

Barbary Macaquen (*Macaca sylvanus)* sind saisonal reproduzierende Primaten welche in „multimale-multifemale" Gruppen leben. Die Weibchen bilden auffallende Schwellungen der Anogenitalregion aus, um ihre Fruchtbarkeit zu signalisieren. Das An- und Abschwellen wird durch Zyklushormone reguliert. Sexuelle Schwellungen haben einen starken Einfluss auf intra- und intersexuelle soziale Interaktionen und zeigen die Attraktivität der Weibchen an. Ob diese sexuellen Schwellungen verlässliche Signale für die fertile Phase der Weibchen sind, bleibt noch unbeantwortet. In dieser Studie wird erstmals die Morphologie dieser Signale mit einer 3D Analyse, mittels fotogrammetrischen, geometrischen und morphometrischen Methoden, untersucht. In 2D Studien werden für die Schwellungberechnungen Längen- und Breitenabmessung der Schwellung von Fotos herangezogen. Dadurch kann allerdings nur eine Flächenbemessung, jedoch keine Volumsberechnung vorgenommen werden. Anhand von Verhaltensdaten, sowie physiologischen und morphologischen Daten wird getestet, ob das männliche Interesse an den Weibchen mit maximaler Schwellungsgröße und Ovulation der Weibchen (gemessen am Verhältnis von Östrogen zu Progesteron (EP)), sowie mit dem weiblichen Verhalten des Präsentierens der Schwellung („female presentation"), einhergeht. Basierend auf Ergebnissen früherer Studien wird erwartet, dass, innerhalb des weiblichen Zyklus, das männliche Interesse an Weibchen („male interest") zum Zeitpunkt rund um die Ovulation erhöht ist wenn die sexuelle Schwellung, verbunden mit gesteigertem EP, vermeintlich am größten ausgebildet ist. An einer Population frei lebender Barbary Macaquen, welche den Fels von Gibraltar bewohnen, wurden Verhaltensbeobachtungen durchgeführt, sowie Urinproben für Hormonanalysen gesammelt und Videosequenzen der Anogenitalschwellungen aufgenommen. Die Ergebnisse zeigen, dass Männchen ein erhöhtes Interesse an Weibchen zeigten, wenn „female presentation" hoch war sowie bei vermehrtem sexuellen Schwellugsvolumen. Allerdings konnte kein signifikanter Zusammenhang zwischen EP und männlichem Interesse festgestellt werden. Aus diesen Ergebnissen zeigte sich, dass „female presentation" und das Volumen der Schwellungen eine wichtige Rolle beim Signalisieren der Fruchtbarkeit und der Attraktivität der Weibchen spielten und daher einen starken Einfluss auf das männliche Interesse hatten. Die fehlende Korrelation zwischen „male interest" und EP steht im Gegensatz zu Studien mit 2D Messungen. Die unterschiedlichen Ergebnisse zwischen 2D und 3D Studien könnten daraus resultieren, dass die 3D Methode spezifischere Information bezüglich weibliche Attraktivität aufzeigt, was nicht unbedingt mit der Ovulation verbunden sein muss.

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