Sexual swelling in bonobos: variations, ovulation predictability, and male responses

Short title: Sexual swelling in female bonobos

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Abstract

Female bonobos exhibit prolonged receptivity with long-lasting maximal swelling phases, which is suggested to lower male mating competition. However, it remains unclear whether easier access to receptive females can reduce male-male competition, given the exclusive nature of male reproductive competition and the skewed paternity toward the alpha male in bonobos. We tested whether wild male bonobos could discern the female fertile phase within the maximal swelling phase using hormonal and behavioral approaches. We found that as the probability of ovulation increased, male mating efforts, including intensive following, solicitation and intervention of copulation, also increased. When multiple females exhibited maximal swelling, males concentrated their mating efforts on the female with older infants and whose maximal swelling phase had started earlier. Although the most frequent copulation was observed between high-ranking males and females with younger infants whose pregnancy probability was low, this result reflects more selective and fewer matings by the females with older infants. We also showed that the low predictability of ovulation can result from large inter- and intra-individual variations in the length of the maximal swelling phase leading to a low day-specific ovulation probability that distributes evenly throughout the whole maximal swelling phases. Taken together, our results demonstrate males' ability to estimate the probability of ovulation from maximal swelling and confirm noisy ovulatory signals in bonobos. Despite the noisy signal from females, our findings highlight that male bonobos can cope with it by adopting a simple but physically demanding mating strategy – concentrating mating efforts on females with older infants throughout the whole maximal swelling phase. Further investigation into the mechanism and evolution of such noisy signals in our closest relatives will not only clarify the function of the erroneous signals, but also help us to re-evaluate the concept and function of so-called concealed ovulation in humans within the evolutionary continuum of different levels of ovulatory signal conspicuity in primates.

Keywords

bonobos, sexual swelling, ovulation, male mating strategy, predictability of ovulation, society

Introduction

The evolutionary function of ovulatory signals and their regulative role in sexual selection has been explored intensively in many primate species [1–5]. Particularly, the exaggerated sexual swelling – conspicuous changes in the size of swelling and color of the anogenital skin in relation to ovulation found in Old World primates – has acquired researchers' attention due to its functional significance for females in balancing between exerting their preference and avoiding the risk from male's coercive mating strategies [1,3,5]. However, we still do not fully understand the degree to which females in a certain species can avoid the risk and take advantage of manipulating male reproductive strategies by controlling the signal conspicuity and reliability [2,4].

Female bonobos (*Pan paniscus*) exhibit exaggerated sexual swelling whose length of the maximal swelling phase (MSP) is the longest among the primates with exaggerated sexual swelling [6–8]. Bonobos are also known for their tolerant and female-centered social characteristics compared to those of male-dominated and aggressive chimpanzee (*Pan troglodytes*) society [9–11]. One proposed mechanism to explain the tolerant and peaceful bonobo society is the estrus-sex-ratio hypothesis [7]. The estrus-sex-ratio hypothesis proposes that the prolonged receptivity of females results in milder mating competition between males and contributes to the peaceful bonobo society. The early achievement of MSP in female bonobos during lactation periods when the infant is still younger than 2 years [12–14], together with MSP during pregnancy [6,15], is therefore assumed to play an important role in easing male-male competition over mates by extending the receptive periods of females. In contrast, female chimpanzees exhibit fewer MSPs between parturition [3,5,16] which makes male-male competition in chimpanzees more severe. Based on this species difference in female receptivity (or availability of receptive females), it is suggested that the primary role of prolonged receptivity in bonobos is not only to confuse paternity certainty but also to reduce the tension surrounding male reproductive competition by allowing males to increase their mating opportunities with females with maximal swelling (FMSs).

The lowest predictability of ovulation from the onset of MSP in bonobos among the species exhibiting exaggerated sexual swelling [4,13,17] seems to be in line with the notion that prolonged ovulatory signaling helps confuse paternity and makes each swelling cycle less attractive to males [2,3,18]. However, it remains uncertain to what extent male mating competition is reduced by easier access to mating due to the greater number of receptive females and low predictability of ovulation from the onset of MSP. The estrous-sex-ratio hypothesis also implies that male-male competition over mates can be eased if they can mate with an FMS as it could increase siring probability. However given prevailing male-male competition over mates [19] and skewed paternity toward highest-ranking males in bonobos [20,21], the regulative role of the higher abundance of FMSs in bonobo society is questionable. Additionally, it is also questionable why females maintain such a costly ovulatory signal by lengthening the period of MSP as it can increase maintenance costs and the probability of sperm competition that can hinder their preference toward high-ranking males [22].

Although studies on wild chimpanzees suggested that female age and reproductive events including lactation and pregnancy are influencing factors for the resumption and continuation of the menstrual and swelling cycle [3,5], previous studies on bonobos have not fully considered those factors [8,13,23,24]. For instance, the MSP may be a less meaningful ovulatory signal for females with younger infants, particularly when the infant is younger than 2 years old [14]. The MSP of such females may also vary in length more than it does for females with older infants making its signal less reliable [25]. Male bonobos may respond differently to the sexual swelling of females with young infants as well, since these females might have a lower probability of becoming pregnant, given the over 4 years of inter-birth interval [7,14].

Despite the lower predictability of ovulation from the onset of MSP in bonobos, about two-thirds of ovulation occurs in the second half of maximal swelling periods [8,14,22,23; *cf.* 13]. If sexual swelling signals ovulation to some degree, male mating activity should have been selected to occur more frequently in the second half of maximal swelling periods, where the fertile window is likely to be. In chimpanzees, male mating efforts, particularly those of the alpha male are concentrated more on the fertile phase of females [4,26,27]. These findings align with predictions from the graded signal hypothesis, which implies that sexual swelling provides a graded signal of the probability of ovulation, distributing males' mating attempts with their social dominance [1,28]. Considering the genetic similarity and mating systems between bonobos and chimpanzees, it is unlikely that bonobo males will not concentrate their mating efforts on females with maximal swelling that signals the fertile phase unless ovulation is independent of maximal sexual swelling.

In chimpanzees, males adopt a coercive mating strategy or mate-guarding to restrict promiscuous mating of females during MSPs [29,30]. The efficiency of male mate-guarding likely depends on the number of males in the group [31] and the degree of ovulation synchrony between females [32,33]. Nonetheless, it is highly beneficial for male chimpanzees if they can determine the fertile window [34] (or peri-ovulatory phase; POP [35,36]) of females within the MSPs, for better reproductive outcomes. In contrast to chimpanzees, male coercive mating has not been documented in bonobos [37]. The high social status of females may also facilitate female choice and prevent males from using a coercive mating strategy in bonobos [10,38]. However, even if their mating strategy differs from that of male chimpanzees, it would be beneficial for male bonobos if they could estimate the probability of ovulation based on sexual swelling and adjust their behavior accordingly. To test the possibility of a male's ability to estimate ovulation probability, we hypothesized that male bonobos could determine the fertile phase of females with some precision – "males know" hypothesis. We defined the fertile phase as a 4-day fertile window, which includes 3 days preceding ovulation and the day of ovulation [36].

Our hypothesis predicts that:

P1: Males will focus their mating efforts on females with higher reproductive quality, determined by the onset of maximal swelling, and the age of the female's infant. Specifically, when multiple females exhibit maximal swelling, males will prioritize females with older infants whose MSP started earlier.

P2: The number of copulations, interventions, and solicitations of copulation by males will increase in correlation with the probability of ovulation and infant age.

P3: Male-male agonistic interactions will escalate when a female nearing ovulation is present in the party, particularly if the female has an older infant. This could result in a higher copulation rate between high-ranking males and females with higher reproductive quality compared to low-ranking males.

Our hypothesis would be supported if male bonobos can discern the fertile phase of female bonobos based on sexual swelling and the infant's age. Therefore, our study will provide an opportunity to examine whether maximal sexual swelling serves as a graded ovulatory signal that exerts influence on male mating efforts in bonobos as in other primates with exaggerated sexual swelling. To test this hypothesis and predictions, we will investigate male mating efforts, including following females, copulations, and aggression, in relation to the probability of ovulation (or maximal swelling) and infant age. After evaluating the relevance of the hypothesis, we will propose a male decision-making rule for mating effort allocation and a possible mechanism that could explain a loose relationship between ovulation and maximal swelling in bonobos. We then suggest that detumescence of maximal swelling could be a key phenomenon for understanding male decision-making, assuming that the mechanism of detumescence in sexual swelling in Old World Monkeys – an increase in serum progesterone concentration after ovulation results in detumescence [39,40] – is likely the same for bonobos [8,14,23]. To test the effect of detumescence on male mating efforts, we used the the calculation of ovulation probability and fertility from the onset of maximal swelling in chimpanzees [36] as done in a previous study in baboons [41]. Finally, based on our findings, we propose and discuss a male mating strategy that can explain how males allocate their mating efforts toward females with different reproductive qualities for a better reproductive outcome.

Results

Males followed females with maximal swelling (FMSs) who had older infants

To test males' ability to estimate ovulation probability, we first investigated the number of males exhibiting intensive following behavior (IFB) after females – following the target female for over 5 minutes within a 10m distance. In total, male IFB after females was observed on 95 days among 250 observation days. The number of males who were in IFB on a day ranged from 0 to 8 (1.4 ± 2.1 males per day). Of these 95 days, there were 6 days when two females were the targets of male IFB. More males exhibited IFB toward the FMS and toward females with an older infant (Fig 1A and 1B; GLMM-1A in Table A in S1 Text). The effect of the number of males and FMSs and the interaction between sexual swelling and infant age were not significant.

<Fig 1. see attached>

Fig 1. Males intensive following behavior (IFB) after females depending on sexual swelling and infant ages. (A) The number of males exhibiting IFB after females increased with the size of sexual swelling. (B) It also increased with

the infant age of females. Sexual swelling was categorized as 1 for non-swelling, 2 for intermediate swelling, and 3 for maximal swelling. Infant age ranges from -1 (pregnancy) to 5. Due to numerous zero values, medians are 0, resulting in a box plot where only a small box representing the 75th percentile and an upper whisker are visible. (C) More males exhibited IFB toward females with older infants and whose maximal swelling was nearing detumescence. The dashed fitted lines were generated by the 'lowess' (locally weighted least squares regression) function in R. (D) The probability of performing IFB by males increased toward females with older infants and whose onset of the MSP started earlier. The dashed fitted lines were generated by logistic regressions. Each dot represents a single data point for each day. Day 0 is defined as the day when the maximal swelling of a given female became detumescent (C) or one day before the onset of MSP (D). (E) The number of males exhibiting IFB after females increased as the female's ovulation approached. Detumescence of maximal swelling started 1.9 ± 2.4 days after the day of ovulation on average. The median number of males in IFB after a given female peaked on the day of ovulation. The box plot represents the 75th and 25th percentiles with a line indicating the median and whiskers representing 1.5 times the interquartile range. The dots represent all data points.

Males focused on a female for IFB whose maximal swelling was nearing detumescence and whose infant was 3 years old or older – a significant interaction term between the two explanatory variables (Fig 1C; GLMM-1B in Table A in S1 Text). The other explanatory variables were not significant. When there were more than 2 FMSs, the probability that males chose females for IFB was higher toward the female with an older infant and whose MSP started earlier (Fig 1D; GLMM-1C in Table A in S1 Text). However, the number of males did not influence male choice, indicating that males do not alter their choice depending on the presence of same-sex competitors, nor do they change their preference toward the second most attractive female. Although female age was significant in this model, it could be due to small variations in female age in the data set (Fig A in S1 Text). We also found, using the 14 ovulation-detected menstrual cycles (Fig B in S1 Text), that the number of males exhibiting IFB after females increased when ovulation was imminent (Fig 1E; GLMM-1D in Table A in S1 Text), and with an increase in females' infant age. The other predictor variables, including the interaction, did not predict the number of males engaged in IFB.

The day-specific probability of ovulation and fertility

The day-specific probability of ovulation and fertility were computed using the 14 ovulatory cycles from 5 females (Fig B in S1 Text). We employed the same equation that has been used in previous studies of chimpanzees and bonobos [13,36]. The day-specific ovulation probability was broadly distributed from the 8th to 27th days from the onset of MSP (Fig 2A). The peak probability was on the 19th day from the onset of MSP. This pattern is similar to that of a previous report on wild bonobos from Lui Kotale [13]. The day-specific probability of fertility – the likelihood that the egg coincides with sperm – did not exceed 0.3 (Fig 2A), which also resembles the previous finding [13].

<Fig 2. see attached>

Fig 2. Probability of ovulation, fertility and male IFB. (A) The day-specific probability of ovulation and fertility from the onset of MSP, and (B) from the detumescence of the maximal swelling. Black closed circles and lines represent the day-specific ovulation probability, and red open circles and lines represent the day-specific probability of

fertility. (C) The number of males exhibiting IFB after females from the onset of MSP is distributed similarly to the probability of ovulation in Fig 2A. (D) The number of males exhibiting IFB in relation to the detumescence of maximal swelling shows a similar distribution as found in the probability of ovulation in Fig 2B. Day 1 is defined as the day of onset of the MSP in Fig 2A and 2C. Day 0 is defined as the day of detumescence in Fig 2B and 2D. The box plot represents the 75th and 25th percentiles with a line indicating the median and whiskers representing 1.5 times the interquartile range. The dots represent all data points.

To investigate the likelihood of ovulation in relation to detumescence and how detumescence impacts male behavior, we also calculated the day-specific probability of ovulation and fertility from the detumescent day [41]. The day-specific ovulation probability was concentrated on the detumescence day and showed a clear peak 4 days before detumescence (Fig 2B), which suggests that progesterone, released from the corpus luteum after ovulation, results in detumescence of maximal swelling [14]. The day-specific fertility exceeded 0.8 from 5 to 4 days before detumescence, then decreased rapidly to zero one day before detumescence. The distribution of the probability of ovulation and fertility, regardless of calculation methods, resembles the distribution of the number of males in IFB of the day. The number of males exhibiting IFB was widely distributed when plotted with the days from the onset of MSP (Fig 2C). In contrast, the number of males exhibiting IFB was concentrated on the days nearing detumescence (Fig 2D). Particularly, the median number of males exhibiting IFB increased until detumescence, and no males followed females after detumescence indicating that males use detumescence as a signal to stop IFB toward the target female. We do not have evidence to suggest that males can predict detumescence and adjust their IFB.

Male mating efforts in response to ovulation and male-male aggression

Males' copulations increased when the ovulation day was imminent (Fig 3A; GLMM-2A in Table B in S1 Text). Infant age had a negative effect – males copulated more with females with younger infants (Fig 3B), and male rank had a positive effect – high-ranking males copulated more (Fig 3C). Although male age had a negative effect on copulation (Fig 3D), female age had no such effect (Fig 3E). The interaction between infant age and days from ovulation was not significant. To better understand the effect of male rank and infant age on the number of copulations, we further investigated males' copulations. The top 3 ranking males accounted for 78.9% of all copulations (165/209), and they copulated with females with younger infants most often (Fig C and GLMM-2B in Table B in S1 Text), which contrasts with skewed IFB to the FMSs with older infants. Although high-ranking males copulated more frequently with females with young infants (low reproductive quality), this reflects fewer matings by the females with younger infants. Due to such skewness in copulation, low-ranking males had fewer chances to copulate even with females of low reproductive quality, which does not support a prediction of the estrus-sex-ratio hypothesis [10]. The lower copulation rate of females with older infants with high-ranking males indicates that females are more selective when they have higher reproductive quality, and/or males interfere with copulations with each other.

<Fig 3. see attached>

Fig 3. Prediction plots of males' copulation, intervention, solicitation of copulation in relation to the ovulation day, infant age, male rank, male age, and female age. (A-E) The predicted copulation rate within a male-female dyad (GLMM-2A). (F-J) The predicted rate of intervention of copulation within a female-male dyad. (K-O) The predicted rate of solicitation of copulation by a male within a male-female dyad. Asterisks (*) on each plot indicate P-value (ns: not significant, *: P<0.05, **: P<0.01, ***: P<0.001).

Interventions of copulation between males increased as a female's ovulation day approached (Fig 3F; GLMM-2C in Table B in S1 Text) and for females with older infants (Fig 3G). High-ranking males intervened more frequently in other males' copulation attempts (Fig 3H). Neither male nor female age predicted the number of interventions (Fig 3I and 3J), suggesting that the intervention rate was not influenced by female rank, even though older females have a higher rank than younger females.

Males solicited copulation more from females whose ovulation was imminent (Fig 3K; GLMM-2D in Table B in S1 Text). The effect of infant age was not significant (Fig 3L). Therefore, the higher copulation rate of females with younger infants (Fig 3B; Fig C in S1 Text) was not due to a higher male solicitation rate toward females with younger infants. Both high-ranking and younger males solicited copulation more frequently (Fig 3M and 3N). Female age was not significant, suggesting that older females are not necessarily more attractive to males which contrasts with male's preference for older females, reported in wild chimpanzees [42].

Finally, the hourly number of aggressions between males on a given male on a day (male aggression rate) increased on the day when male IFB was observed and with an increase in the number of males (Fig 4A; LM-2E in Table B in S1 Text). Although the male aggression rate appeared to increase with the number of FMSs on the day (Fig 4B), this trend was not significant. However, there was a significant interaction between the number of males and FMSs on the male aggression rate. To better understand the interaction, we visualized the effect of the number of FMSs and males on the male aggression rate. As shown in Fig 4C and 4D, the male aggression rate decreased when there were more males and FMSs in the party. However, when there were a fewer number of males in the party, the effect of the number of FMSs on the male aggression was opposite so the male aggression rate increased with an increase in the number of FMSs. These results demonstrate that the effect of the availability of receptive/attractive FMSs on the male aggression rates changes depending on the number of males in the party.

<Fig 4. see attached>

Fig 4. The number of male-male agonistic interactions in relation to FMSs. (A) A higher rate of agonistic interactions per hour between males was observed when males exhibited IFB. The presence of IFB is denoted by 'O' and its absence by 'X'. (B) The number of male-male agonistic interactions appears to increase with an increase in the number of FMSs. However, this trend was not significant. (C) The effect of the number of males and FMSs on the hourly male aggression rate based on the model prediction of the LM-2E shows an interdependency between the number of males and FMSs. This plot was redrawn to fit to the frame based on an interactive 3D plot available at https://figshare.com/s/6c01c43ecb3e05ffabd8. (D) Visualization of the interdependency between the number of males and FMSs on the male aggression rate (significant interaction in LM-2E) shows that when there were a greater number

of males in the party e.g., Mean + 1SD (9.6 males in D), the male aggression rate decreased as the number of FMSs increased. However, when there were a fewer number of males in the party, the male aggression rate increased with an increase in the number of FMSs. Each dot represents a single data point, specifically the aggression rate per hour over 250 days. The box plot represents the 75th and 25th percentiles with a line indicating the median and whiskers representing 1.5 times the interquartile range.

Discussion

We showed that male mating efforts were skewed toward females with higher reproductive quality – females with older infants (3 years or older) and those whose maximal swelling was close to detumescence. Males, regardless of rank, exhibited IFB toward females with higher reproductive quality. When there were more than two FMSs, males followed females with older infants whose MSP started earlier, demonstrating males' preference for FMSs with older infants. Although high-ranking males copulated more frequently with females who had younger infants, they did not solicit copulations from females with younger infants. Additionally, high-ranking males intervened in other males' copulation attempts with females with older infants. These results indicate that male-male competition over females with older infants is more intense, resulting in a lower frequency of copulation for females with older infants. Alternatively, females with higher reproductive quality may mate more selectively. Contrary to a prediction from the estrus-sex-ratio hypothesis, male-male aggression increased when the number of FMSs increased. However, such an effect of the FMSs on male-male aggression was reversed when there was a greater number of males than the average number of males in the party, implying that the effect of the availability of FMSs (receptive females) on male-male agonistic interactions is not simple and necessitates further investigation.

One important finding of our study is the correlation between female reproductive quality and male mating efforts, suggesting males' discrimination of female reproductive value based on sexual swelling and reproductive event (e.g., infant age). The male mating strategy we propose later in the discussion – concentrating their mating efforts on the FMSs with older infant and maintaining their efforts until the end of MSP – hints at how the skewed paternity toward high-ranking males in bonobos is achieved [20,21,43] where the predictability of ovulation from the onset of MSP is the lowest [26,44,45]. The social and physical ability of high-ranking males to outcompete others may cause a reproductive skew toward them in bonobos. However, given the high female social status and female choice, male mating monopolization is not solely down to males' competitive ability, but also to female preference for high-ranking males with high durability as high-ranking males need to pursue mating efforts for over 10 days per MSP of each female. There may also be another female counterstrategy (adaptation) against males' monopolizing mating strategy. At Wamba, two females often gave birth within 2 weeks (Table C in S1 Text), suggesting a loose synchrony in menstrual cycles that includes overlapping MSPs, but not the 4-day fertile windows. Such a loose synchrony allows females to test males' physical durability in maintaining mating efforts, including sperm quality, for longer periods, and reduces female-female competition over potential mate by avoiding an overlap of the fertile window. If the loose synchrony does not happen by chance, this will serve as a counterstrategy to males'

monopolizing mating strategy by enabling females to bet on male durability along with male competitive ability. Unfortunately, it was not possible to test whether those females who gave birth closely shared the same father as Ki was the alpha male's mother and a paternity test has not been conducted for No and Ot's offspring born in April 2015. We can also recall the estrus-sex-ratio hypothesis in that a greater number of receptive females whose menstrual cycles are loosely synchronized can have a regulative role in male-male competition. Contrarily, tight synchrony leading to an overlapping fertile window may cause a competitive situation among females, which is not beneficial for females and may not be the case in bonobos given the very skewed paternity.

One puzzling trend in copulation in this study is that high ranking males accounted for most copulation even with FMSs with younger infants (Fig C in S1 Text). This trend can be due to the lower copulation rate in FMSs with older infants as they are more selective, accepting fewer copulation attempts with their increasing possibility of pregnancy and the opposite behavioral response of the FMSs with younger infants. It is questionable why high-ranking males spend their energy and time engaging in copulation with females with low pregnancy probability. If their sperm is limited, they would allocate it more prudently. However, if ejaculations involved in copulation can help with refreshing sperm quality, their behavior is reasonable. Regardless of the adaptive value of this behavior, this copulation trend suggests that low ranking males do not have much chance for copulation even with FMSs with younger infant, questioning the prediction of the estrus-sex-ratio hypothesis. To better understand the effect of the higher availability of receptive females (FMSs) on the peaceful bonobo society, further investigation is necessary on the extent to which mating with FMSs can reduce or ease male-male competition over mates.

In this study, we showed that most ovulations occurred in the last half of the MSP (Fig B in S1 Text). The day-specific ovulation probability on a given day was also low and widely distributed from the onset of MSP (Fig 2A) as in a previous study [13]. How can we integrate such a wide distribution of day-specific ovulation probability and the concentrated occurrence of ovulation toward the end of MSP? We propose that inter- and intra-individual variations in the length of MSP are the key reason (source of noise) why the day-specific probability of ovulation calculated from the onset can be low in bonobos. The equation (see methods) calculates the day-specific probability of ovulation by summing up all probabilities of a given day from all defined ovulatory MSPs in a study population. Therefore, there could be two ways to achieve a wide distribution of the day-specific ovulation probability. One is that ovulation occurs randomly within the MSP. The other is that even if ovulation occurs at the end of MSP, large inter- and intra-individual variations in the length of MSP result in a wide distribution of the day-specific ovulation probability. The standard deviation of the length of MSP seems to vary more greatly in bonobos than chimpanzees (Table D in S1 Text), although we could not conduct a statistical test to compare whether the variation in bonobos is larger due to the lack of raw data from the published works. However, the length of MSP is longer in bonobos than chimpanzees (Mann-Whitney U Test; $W = 48$, $p = 0.024$), suggesting a greater possibility of wider day-specific ovulation probability distribution across the MSP as larger mean values likely coincide with greater variations [2]. If this is the case, the poor predictability of ovulation from the onset of MSP does not

326 necessarily mean that ovulation more randomly deviates from the MSP in bonobos. Instead, it is the inter-
327 and intra-individual variations in the length of MSP causing the low predictability of ovulation from the
328 onset of MSP and its wide distribution across MSP. There is a greater variation in the length of the follicular
329 phase compared to the luteal phase, both between and within individuals across menstrual cycles in primates
330 [46]. Among primates, bonobos seem to exhibit the largest individual variations in the length of the follicular
331 phase [2]. As their follicular phase corresponds to MSP [14,23], the variation in the follicular phase length
332 would inevitably lead to variation in the length of MSP. This, in turn, leads to greater difficulty in predicting
333 ovulation from the onset of MSP in bonobos as the equation sums up all MSP durations from all females in a
334 study group to calculate day-specific probability of ovulation. However, the equation-derived low
335 predictability of ovulation does not necessarily imply males' great difficulty in predicting ovulation as
336 detumescence can provide a post-hoc signal for the occurrence of ovulation. Males indeed can deal with such
337 a low predictability of ovulation by matching their mating efforts near detumescence of MSP (Fig 2D).
338 Given the highly skewed paternity toward high-ranking males [20,21,43], the male's strategy of allocating
339 mating efforts to females with old infants and the detumescence of the MSP, therefore, will be a successful
340 mating strategy that guarantees male reproductive success in bonobos, even if female choice can also be an
341 influencing factor on male reproductive success.

342 Sexual swelling in bonobos seems to be located on one end of the spectrum among the primate species with
343 sexual swelling in that they exhibit the loosest relationship between ovulation and maximal swelling [13].
344 However, we do not yet know the physiological mechanisms that cause such a large variation in bonobos.
345 While it is important to explore mechanisms that increase random errors in the ovulation occurrence during
346 MSP or that prevent the causal relationship between hormones and sexual swelling, the hormonal mechanism
347 that controls ovulation and the occurrence of sexual swelling in bonobos may not deviate much from
348 chimpanzees given their genetic similarity [47–50] and the relationship between sex-steroid hormones and
349 swelling [14,23]. Instead of suggesting an error-introducing mechanism, we propose that reproductive
350 events, including lactation, pregnancy, and early miscarriage [51], along with the possibility of higher
351 estrogen sensitivity in female bonobos [14], can cause significant variations in the length of MSP. These
352 factors can introduce instability to the follicular phase length, leading to low predictability of ovulation from
353 the onset of MSP. For example, as depicted in Fig D in S1 Text, eight days of post-conception swelling were
354 observed right after implantation in a female named No, within 3 weeks of ovulation (fertilization). This
355 post-conception swelling is likely due to an estrogen surge preceding a progesterone surge after implantation
356 [52], resulting in a similar estrogen/progesterone ratio at the onset of MSP. This is also a plausible
357 mechanism for post-conception MSP in bonobos as in other primates [52–54]. Moreover, if an early loss
358 happens after the post-conception MSP, as found in No (Fig D in S1 Text), this post-conception MSP could
359 be defined as an MSP that deviated from ovulation. Male bonobos might be misled by such post-conception
360 swelling, as losing a siring chance is costlier than allocating mating efforts, even for the post-conception
361 MSP [55]. If the early miscarriage frequency in bonobos is similar to that in humans (over 20% [51]), the
362 early miscarriage could cause the timing of MSP to seemingly deviate from ovulation. Therefore, in
363 conjunction with the potential high sensitivity of bonobo sexual swelling to estrogen [14], physiological

variations from reproductive history might be key sources of the noise causing inter- and intra-individual variations in the length of MSP. Further studies on molecular-level hormonal sensitivity of sex skin and hormone–swelling dynamics induced by fertilization events will help elucidate the source of this noise and enhance our understanding of the mechanism and evolution of such presumably noisy ovulatory signal in bonobos.

Sexual swelling is a graded-signal indicating the probability of ovulation in many primate species [17,36,55,56]. Even if the predictability of ovulation from the signal seems to be the lowest in bonobos [13,14,17], detumescence can be a reliable sign of ovulation that predicts male mating efforts (Fig 2B, 2D). Although male bonobos may have the ability to discriminate subtle changes in the size of sexual swelling as seen in chimpanzees and barbary macaques [26,57], if male bonobos simply maintain or gradually increase their mating efforts until swelling detumescence, they can cope with the low predictability of ovulation, regardless of their ability. This simple male behavioral strategy will help male bonobos deal with the lowest predictability of ovulation, although the energetic and physical costs involved in this strategy are not cheap. Despite the stochastic risk of missing siring opportunities involved in this strategy, given paternity data in bonobos and high ovulation predictability based on detumescence (Fig 2B), the cost of discontinuing their mating effort after detumescence would be very low. This strategy will also be effective in other primate species where females exhibit any graded-signals, including changes in skin color, size, or odor in relation to ovulation [55]. Further investigation with precise measurement of any ovulatory signals will clarify whether males indeed discriminate subtle changes in the ovulatory signals or adopt a simple but physically demanding mating strategy as male bonobos did in this study. This will provide a valuable opportunity to better understand how males have evolved to recognize and respond to females' signaling of ovulation probability and integrate the concept of concealed ovulation in humans into an evolutionary continuum of ovulatory signal conspicuity within the primate taxa.

Materials and Methods

Study site and subjects

We conducted this study on a fully habituated, free-ranging wild bonobo group, E1, at the long-term bonobo field site at Wamba (00˚ 11′ 07.6″ N, 022˚ 37′ 57.5″ E; WGS84) in the northern sector of the Luo Scientific Reserve, D. R. Congo [12,58]. The group's home range includes primary, secondary, and swamp forests. Despite the absence of a clear division of dry and wet seasons, rainfall and fruit availability are lower in January and February [59]. During the study periods from 2013 to 2015, the E1 group consisted of 32 to 38 individuals, including 9 adult and 3 adolescent immigrant females, and 7 adult and 4 adolescent males. Age classes were defined following a previous study [60]. We collected focal data on 9 adult females and 7 adult and 3 adolescent males (Table C in S1 Text). All adult females had immigrated into the E1 group and had at least one successful birth [61].

Behavioral observation

We collected behavioral data on adult males and females during three study periods: SP1 (September 2013 to February 2014), SP2 (July 2014 to September 2014), and SP3 (November 2014 to April 2015). We followed bonobos from their bed site, located at around 6 AM, until they made a new night bed, usually after 5 PM. When the group split into several parties, the largest party was followed. To control for the effect of the number of available group members, we recorded party composition every hour using the one-hour party method [62]. Daily ranging party size and membership were defined as the number and identities of all individuals found during a given day when the total observation time exceeded 2 hours.

For continuous focal sampling, we randomly selected one bonobo from the party based on a pre-generated random order before fieldwork began. We searched for the first target focal animal in the random order for at least 30 minutes after locating the bonobos in the morning. If we could not find the first one, we chose the second one in the order, and so on. To compensate for the lack of focal sampling data due to the species' fission-fusion social system [63], we prioritized individuals with fewer data for focal animal selection. One focal session continued for 20 minutes and was terminated if a focal animal was out of sight for more than 5 minutes within a session. Only focal sessions that lasted longer than 15 minutes were included in the data analysis. After an individual was followed once, it was not followed again for another focal sampling until at least 100 minutes had passed. During a focal session, we recorded all activities involving the focal animal, including feeding, moving, resting, and social interactions. We recorded the names and behaviors of neighboring individuals within 5m of the focal animal at 5-minute intervals, including a scan at the beginning of the focal session. We also recorded all rare events, such as sexual and agonistic interactions, as far as possible. We recorded the individual ID of the participants of the interactions as well as the time and duration. All copulations, genito-genital rubbings, and agonistic interactions (e.g., bite, hit, displacing, and chase) were recorded whenever observed.

Ejaculation during copulation was not always possible to confirm. Therefore, complete heterosexual intercourse without any interruption by others was counted as copulation. Intervention in copulation included direct aggression toward a male who solicited copulation from a female, and direct intervention that targeted the male during copulation. Intervention directed toward females from males was not included as there were only 22 attempts and most of them led to counter-aggression from females or were otherwise ineffective. Only 5 cases of intervention directed toward females were successful in stopping copulation. Solicitation of copulation was counted only when a clear target was identified. Male bonobos often have an erect penis during feeding while watching females, so only the presence of an erection was not counted as solicitation. For solicitation, an erection should coincide with other behaviors such as directed hand or body gestures [64] or shaking, bending, or dropping branches or twigs to get attention from the target female. We also recorded agonistic interactions whenever there was a clear target, regardless of physical contact. Therefore, undirected display behaviors, such as charging and branch dragging, were not considered agonistic interactions [65]. When a counterattack was performed by the target within 5 seconds of the directed aggression toward the target, we considered it a tied interaction.

To evaluate males' interests toward females in the party, we defined male IFB (intensive following behavior) after females as when a male maintains proximity (within 10m maximum) to the target female for more than 5 minutes while moving, without losing the target female from his sight. If a male's following behavior met these criteria, we considered that this male performed IFB toward the target female.

In total, we followed bonobos for 1462.3 hours over 255 days (5.73 ± 1.25 hours per day). The total focal sampling time for all individuals was 280.22 hours (858 sessions: 14.75 ± 2.01 hours per individual). The average length of a focal session was 19.6 ± 1.0 minutes, and each session consisted of 4.88 ± 0.34 scans (4189 scans in 858 sessions). We excluded two days from the 255 days when we were able to follow bonobos for less than 1 hour. Additionally, one day with very heavy rain when no males were confirmed, and two days when we could not see any females were removed from data analysis, except for the calculation of male dominance relationships.

Assessment of sexual swelling and the MSP

We scored the daily variation in sexual swelling of female bonobos in relation to the firmness and size of each individual's swelling [6,66]. The sexual swelling status was assigned to one of three categories. Fig E in S1 Text shows an example of changes in sexual swelling of one individual, named Fk. Swelling 1 is for non-swelling status; swelling 2 is for intermediate swelling; swelling 3 represents maximal swelling. Unlike female chimpanzees, the sexual swelling of female bonobos is always visible, even in the non-swelling status. Therefore, the practical definition of non-swelling status (swelling 1) is that the swelling is within its minimum size range. The swelling size variation of an individual between swelling 2 and 3 was sometimes not definitive. Therefore, firmness and shininess of the surface and movement of the sexual swelling during locomotion were the key features for distinguishing swelling 2 and 3. The detumescence day was set to the first day when the maximal swelling started shrinking, lost its firmness and shininess, and the swelling score moved from 3 to 2. The MSP was defined from the first day of the appearance of the maximal swelling to the end day of the MSP before detumescence. If sexual swelling recovers from 2 to 3 within 4 days, it was considered that the MSP was continuous, following the previous study [13]. To reduce observation errors and bias in sexual swelling scoring, at least one researcher and two research assistants discussed the sexual swelling status of each female during field observations and decided scores at a daily meeting every evening.

Urine sample collection and hormonal measurement

Urine samples were collected using filter papers (Whatman #1 Ø 5.5cm) throughout the day. After a female finished urination, we collected urine from the leaves of terrestrial plants or small trees. We placed the edge of the filter paper (around 1/4 of the whole) into the urine droplets and waited until it absorbed around 2/3 of the paper, allowing us to avoid contamination from dirt on the leaves. To prevent disturbing the bonobos, we collected urine after they had left the area, maintaining a minimum distance of 5m. We did not collect urine that was mixed with feces or other bonobos' urine. Upon returning to camp, urine-soaked papers were placed in a dry box containing 500g of silica gel. Although the papers typically dried within 2 days, they were kept in the dry box for a minimum of 5 days. The silica gel was replaced weekly and heated in a hot pan for over

30 minutes for reuse. Once each paper was removed from the dry box, it was individually packed in plastic zipper bags, then placed in a larger zipper bag with silica gel and stored in a dark room. The longest period that the sample was stored at room temperature was 6 months. After the samples were transported back to Japan, they were stored in a freezer (-20°C) until the time of urine extraction. A previous report demonstrated that using this method, the estrogen and progesterone metabolite levels do not change for 6 to 12 months even at room temperature [67,68].

We used an enzyme immunoassay (EIA) to measure urinary metabolites of estrogen (E_1C) and progesterone (PdG). In total, we successfully recovered urinary E_1C and PdG from 660 urine samples from 9 females, which were used to determine ovulatory swelling cycles (Table C in S1 Text). For the EIA, urine was extracted from the filter paper using deionized water with mechanical shaking [68]. Following extraction, we measured the creatinine concentration of the samples using the Jaffe reaction [69]. When the creatinine concentration of the samples exceeded 3mg/dl, we retested them by diluting the extracted samples. Urinary E_1C and PdG concentrations were measured by EIA, as described in a previous study [70]. We used antibodies (Cosmo Bio in Japan) against estrone-3-glucuronide BSA, pregnanediol-3-glucuronide BSA, and horseradish peroxidase conjugated steroid derivatives (Cosmo Bio in Japan). More details regarding the EIA are published elsewhere [68,71]. The sensitivity of EIA was 6.6pg/ml for E_1C and 2.1ng/ml for PdG. If the concentration was below these values, we excluded that sample from the analysis. The inter-plate CV for E_1C was 10.79 and 15.83 for PdG. The intra-sample CV was 5.66 for E_1C and 6.66 for PdG. ANCOVA tests with different dilutions from three different bonobo urine samples (N = 3 x 4) confirmed that there was no violation of parallelism between the diluted samples and the standards (*P* values ranged 0.155 to 0.337 for E_1C and 0.255 to 0.730 for PdG). The recovery test, which involved adding a fixed amount of standard solutions to three different samples (N = 3 x 4), showed high regression coefficient values (E_1C: $Y = 0.99X + 0.01$, $r^2 = 0.985$, PdG: $Y = 0.99X - 2.5$, $r^2 = 0.967$), suggesting that E_1C and PdG were successfully recovered in assays.

Defining the fertile window and probability of ovulation and conception within a swelling cycle

The date of ovulation was estimated based on a sustained rise in urinary PdG above the baseline [14]. Briefly, the baseline was defined as the mean of urinary PdG concentration of the 10 days preceding a given day. If the PdG concentration on a given day exceeded two standard deviations above the baseline for three consecutive days within a week, the day before the first day of the sustained PdG rise was considered the ovulation date. We used a 4-day fertile window, also known as the periovulatory period [36], starting from three days prior to ovulation, based on findings that sperm remain fertile within the reproductive tracts for three days and eggs remain fertile within 24 hours after ovulation [72,73]. This criterion has been used in several studies on various primate species, including wild chimpanzees in Taï [36] and wild bonobos in Lui Kotale [13].

To calculate the probability of ovulation on a specific day from the onset of the MSP, we used the following equation proposed in a previous study [36].

$$P(T = t) = \frac{n_t}{n}, t = 1,2,3\ldots$$

In this equation, $P(T = t)$ represents the probability of ovulation on a given day. Here, t denotes a specific day within the MSP, which starts from the onset of MSP. n_t is the total number of cycles in which ovulation occurred on day t and n is the total number of ovulatory cycles, which is 14 in the current study (Fig B in S1 Text).

We also calculated the daily probability of fertility (the likelihood that the sperm cell meets the egg; fertilization) using the equation from the same study [36].

$$P(X(f) = 1) = \sum_{t=f}^{f+3} P(T = t)$$

In this equation, $P(X(f) = 1)$ represents the probability of fertility (fertilization) on a given day within the MSP. More simply, the probability of fertilization on a given day is the sum of the probability of ovulation on the given day f and the following 3 days from the given day f, as calculated in the previous equation for the probability of ovulation. By using this equation, we can directly compare our results between species, as well as between populations of the same species.

Estimating male dyadic dominance hierarchy

We assessed male dominance relationships based on dyadic agonistic interactions. Agonistic interactions with physical contact included biting, hitting, and trampling, while those without physical contact included chasing, charging, and directed display (sometimes with branch dragging). We only used dyadic agonistic interactions that had a clear outcome of loser and winner. A loser was defined as the recipient of aggression who exhibited submissive behaviors such as grimacing, screaming, running away, or retreating. We excluded tied agonistic interactions from the calculation of relative dominance. In total, we observed 844 dyadic agonistic interactions. Only 16 dyadic interactions were followed by counter-aggressions. Therefore, we selected 828 dyadic interactions (SP1: 314, SP2: 162, SP3: 352) to calculate the male dominance hierarchy (Table E in S1 Text). We used the steepness package [74] to build a dominance hierarchy and calculate David's score. We also checked the h′ index [75], a modified version of Landau's linearity index [76], using the igraph package [77] to determine whether the male dominance hierarchy was linear.

Statistical analysis

We used R 4.3.3 [78] and several packages including lme4 [79], lmerTest [80], car [81], ggplot2 [82], and sjPlot [83] for statistical analyses and graphics. We used generalized linear mixed models (GLMM) or linear models (LM) with negative binomial and binomial distributions. Mixed models that included random effects were used to control the repeated measures of behavior from the same individual. We also checked multicollinearity between independent variables in the model based on variance inflation factors (VIFs) after we ran models. In all models, the maximum VIF was less than 3, except for the non-significant interaction

term in GLMM-1A, which had a VIF of 3.44. This indicates that multicollinearity among the predictor variables is not a serious concern [84]. All models were also significantly better in explaining the data compared with null models.

We considered that the reproductive quality of female bonobos has two main components. One is the increased chance of pregnancy as infants grow over time, irrespective of the menstrual cycle on a broad timescale. The other is changes in the probability of fertility (the chance of fertilization – a sperm meets an egg) within a menstrual cycle, depending on changes in the probability of ovulation over time. In chimpanzees, these two components are likely closely related because the energetic constraint from lactation plays a regulatory role in the resumption of the menstrual (swelling) cycle [16]. This is probably similar to female bonobos, although bonobo females resume their swelling cycle much earlier [7,14]. To integrate the effect of a dependent infant on female reproductive quality, we used infant age as a proxy for female body condition. To examine the effect of ovulation, we also included the number of days from ovulation, detumescence, or the onset of MSP as a predictor variable in each model, depending on our question. The day of ovulation (or detumescence) was coded 0, one day before ovulation was -1, two days before was -2, and so on. The days after ovulation were 1, 2, and so on. The onset of MSP was coded as 1, the next day was 2, and so on. In some analyses, we confined the data from -14 days before ovulation to the ovulation day (15 days, -14 to 0) to control for large inter- and intra-individual variations in the length of the MSP. This 15-day-length MSP was selected as the mean length of the MSP from a published study was 13.5 ± 1.8 days (Table D in S1 Text).

Infant age was coded on an ordinal scale by grouping 6 months as 0.5 years. It ranges from -1 to 5. From six months before parturition to the day of parturition was coded as -0.5 and the newborn infant was coded as 0. We also coded -1 from the detumescent day of maximal swelling which coincided with ovulation resulting in successful deliveries of an infant. Finally, to avoid high VIF and convergence errors of the model, we standardized all predictor variables to make all variables have a mean of 0 and standard deviation of 1. To make figures more intuitive and easier to interpret, they were made with the original scale.

To investigate the factors influencing a male's IFB toward a certain female, we ran two GLMMs with a negative binomial distribution, following the hurdle model approaches [85]. In the first model (GLMM-1A in Table A in S1 Text), we tested whether swelling status and infant age (explanatory variables) predict the number of males (response variable) in IFB toward a specific female using all data (250 days). Female age was also included as a fixed effect to examine its influence on male IFB. The number of males and females with maximal swelling (FMSs) were also included as fixed effects to examine whether the spatio-temporal distribution of males and FMSs on a given day influences the number of males who exhibit IFB. The interaction between swelling status and infant age was also included and female ID was the random factor in the model.

In the second part of the hurdle model, we took a subset of the data, which consisted only of those FMSs (205 days), to examine the effect of detumescence of maximal swelling and infant age (GLMM-1B in Table A in S1 Text). In this model, the days from detumescence of maximal swelling were included as an

explanatory variable instead of swelling scores. The other variables were the same as in GLMM-1A, except for the inclusion of the interaction between detumescent day and infant age.

We also investigated how males chose the target of the intensive following using a binomial mixed model (GLMM-1C in Table A in S1 Text) with the data when there were more than 2 females with maximal swelling in a daily ranging party (77 observation days). In this model, the number of days from the onset of MSP and infant age, as well as their interaction, were included as explanatory variables. Female age and the number of males of the day were also included as in the two previous models.

Finally, using the data from the 14 ovulation-detected menstrual cycles (138 observation days), we tested the effect of ovulation on male IFB toward females using a negative binomial GLMM (GLMM-1D in Table A in S1 Text). In this model, other explanatory variables were identical to GLMM-1B except that we changed days from detumescence to days from ovulation (ovulation day was coded 0).

To investigate the factors influencing male behaviors, including copulation, intervention in copulation, and solicitation of copulation, which are directly related to male mating efforts, we conducted four separate GLMMs with negative binomial distribution. For these analyses, we used data from the 14 ovulation-detected menstrual cycles and limited the data from -14 to 0 days from ovulation (104 days in total).

Each model (copulation: GLMM-2A and 2B, intervention: GLMM-2C, solicitation: GLMM-2D in Table B in S1 Text) uses the number of copulations, interventions, and solicitations of copulation observed within a male-female dyad on a given day as a response variable. The number of events was adjusted using the offset function, which included the hours that the male and female in the given dyad were observed together in the same party on a given day. The explanatory variables were the number of days from ovulation, infant age, male rank, male age, and female age. In GLMM-2B, male rank was categorized into two groups, high (1st to 3rd ranking) and low (4th to 10th ranking), to further investigate the effect of a 3-way interaction between days from ovulation, infant age, and male rank on the number of copulations. The female-male dyad nested within a certain menstrual (swelling) cycle was integrated into all models as a random variable (coded as dyad ID: cycle name).

To test whether the number of FMSs and males influences the hourly male-male aggression rate, we ran an LM (LM-2E in Table B in S1 Text). As male-male aggression might be related both directly and indirectly to male mating competition, we included the presence of male IFB (O or X) as a fixed effect, along with the number of FMSs and males on a given day. To calculate the male-male aggression rate per male per hour, we divided the total number of male-male aggressions on a given day with the total following hours of the day. We then conducted a square root transformation of the aggression rate to avoid a violation of the normality assumption of the model. We used an LM, not GLMM, as the random effect could not be defined since the number of male-male aggression incidents was pooled within a day.

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Data and materials availability

All data for statistical models and figures, including R codes, will be available from the figshare database after acceptance.

Declaration of interests

The authors declare no competing interests.

Supporting information captions

S1 Text: Supplementary Figures (A to E), Tables (A to E) and References

Figures

Fig 1.

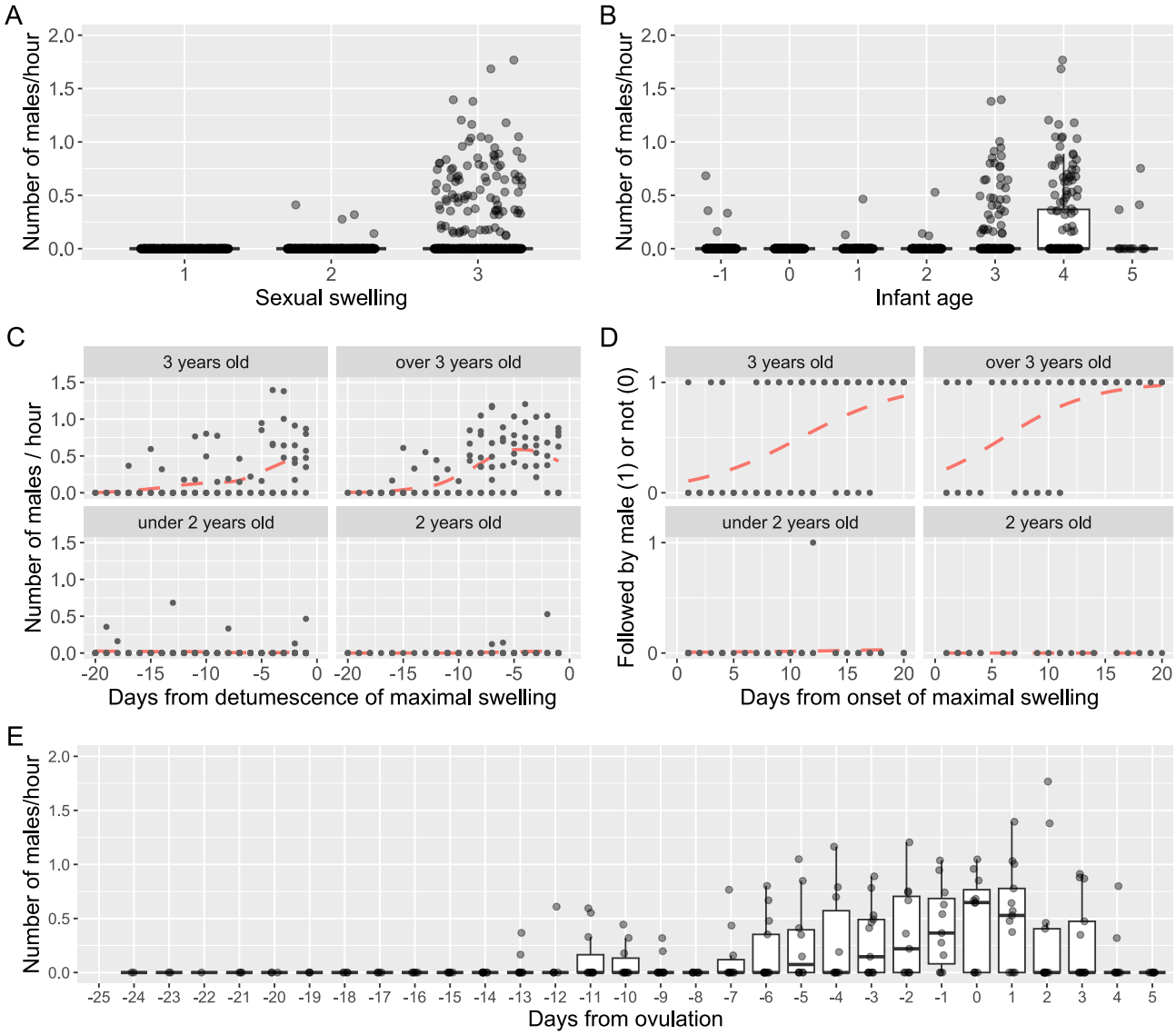


Fig 2.

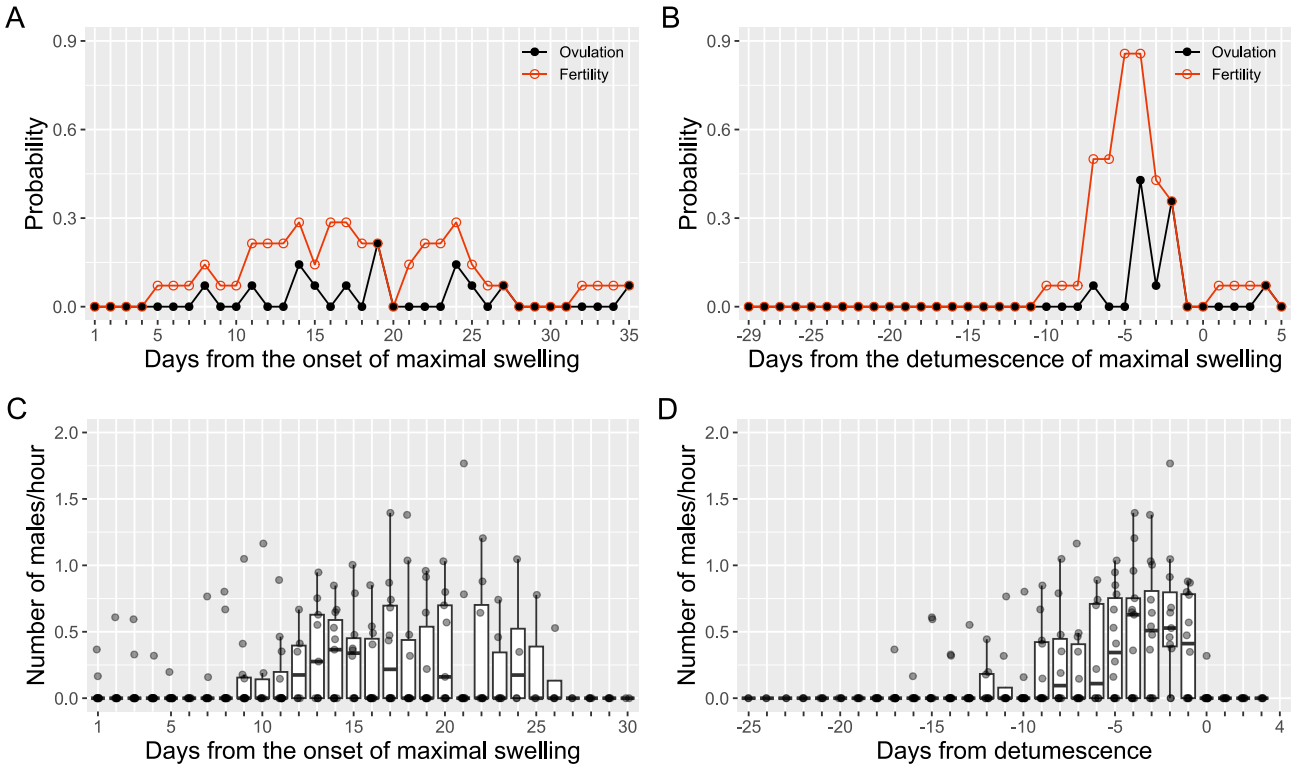

A
Probability
Ovulation
Fertility
Days from the onset of maximal swelling
B
Probability
Ovulation
Fertility
Days from the detumescence of maximal swelling
C
Number of males/hour
Days from the onset of maximal swelling
D
Number of males/hour
Days from detumescence

Fig 3.

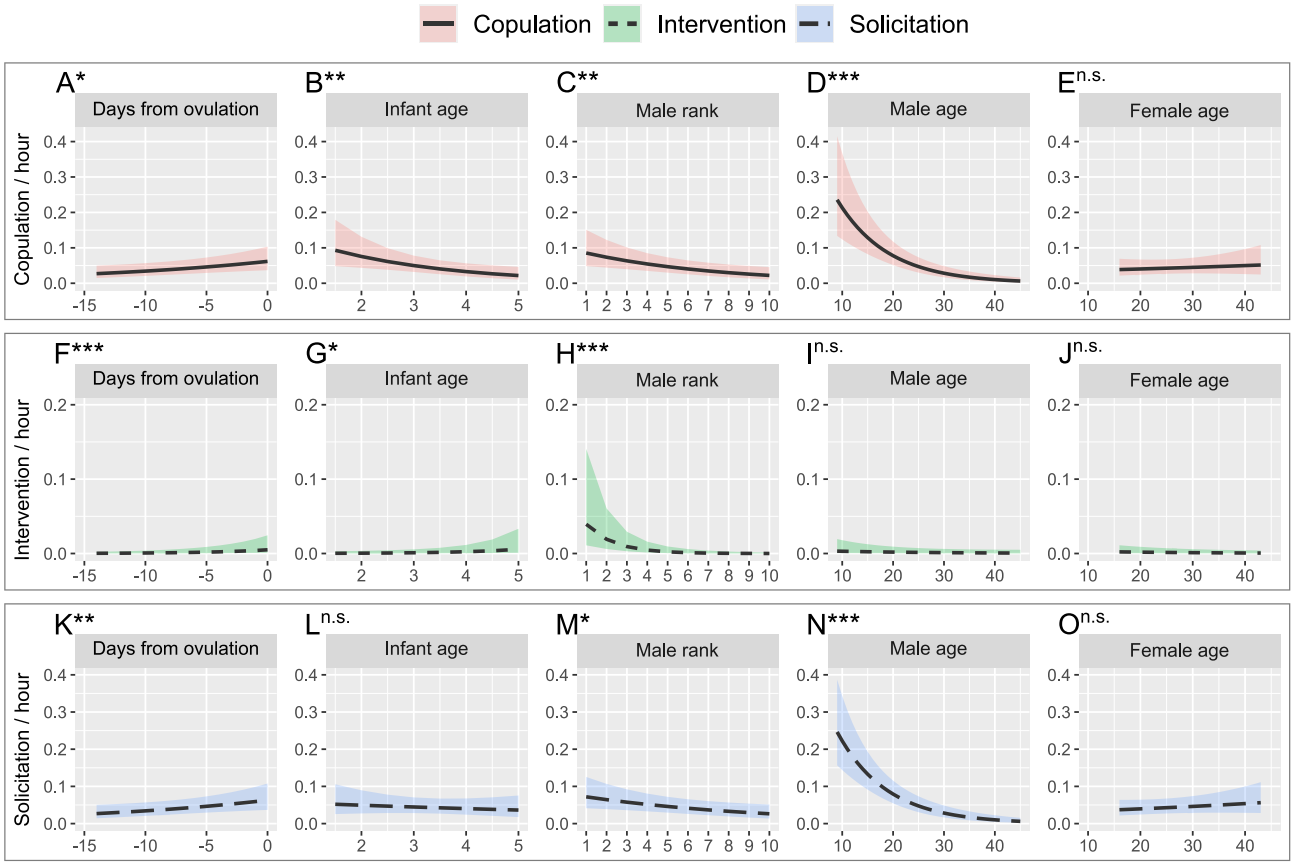
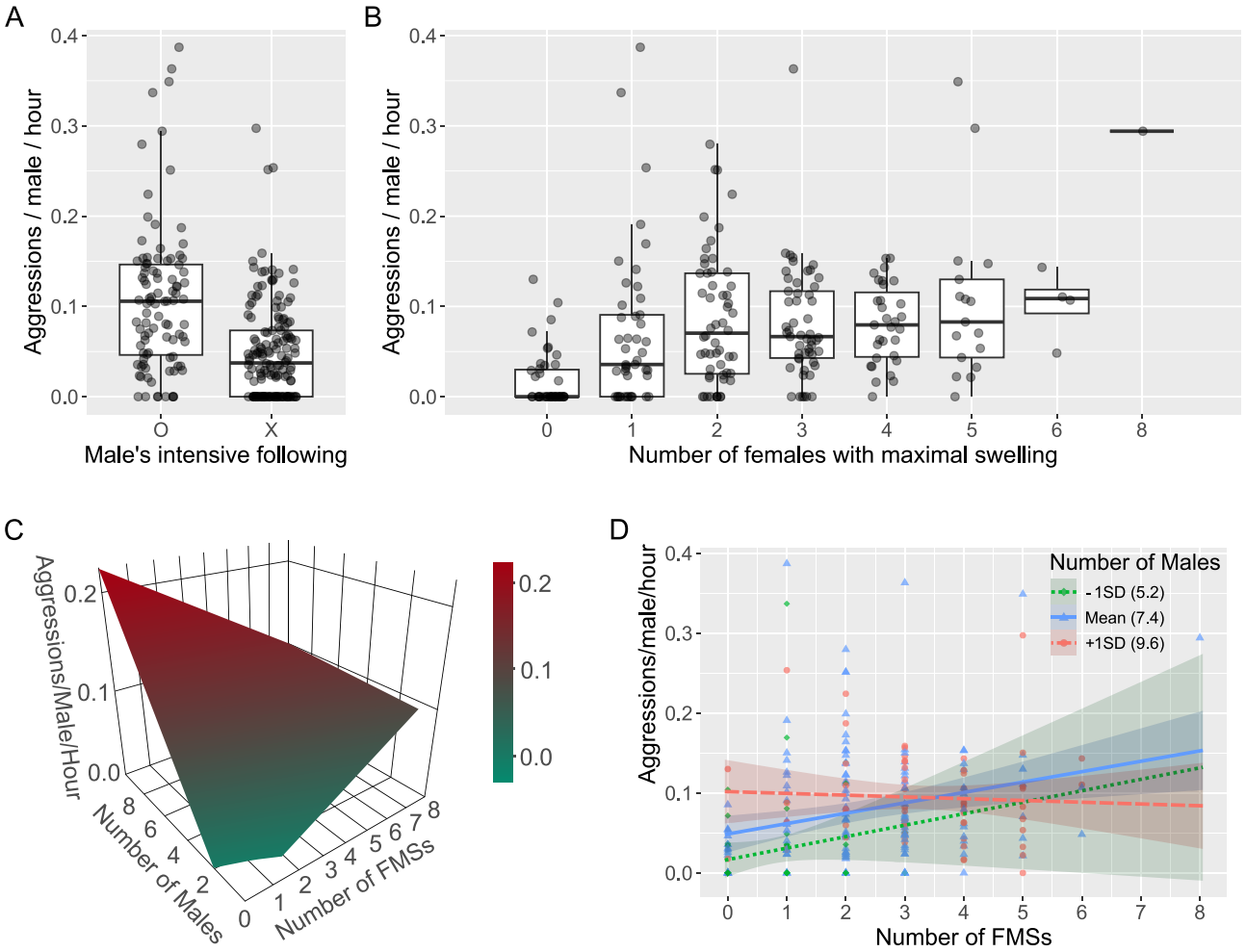

Copulation
Intervention
Solicitation
A*
Days from ovulation
B**
Infant age
C**
Male rank
D***
Male age
E n.s.
Female age
Copulation / hour
F***
Days from ovulation
G*
Infant age
H***
Male rank
I n.s.
Male age
J n.s.
Female age
Intervention / hour
K**
Days from ovulation
L n.s.
Infant age
M*
Male rank
N***
Male age
O n.s.
Female age
Solicitation / hour

Fig 4.


A
Aggressions / male / hour
0.4
0.3
0.2
0.1
0.0
O
X
Male's intensive following
B
Aggressions / male / hour
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
8
Number of females with maximal swelling
C
Aggressions/Male/Hour
0.2
0.1
0.0
Number of Males
8
6
4
2
0
Number of FMSs
1
2
3
4
5
6
7
8
0.2
0.1
0.0
D
Aggressions/male/hour
0.4
0.3
0.2
0.1
0.0
Number of Males
-1SD (5.2)
Mean (7.4)
+1SD (9.6)
0
1
2
3
4
5
6
7
8
Number of FMSs