Mechanisms and socio-sexual functions of female sexual swelling, and male mating strategies in wild bonobos

A Dissertation
Presented to the Graduate School of Science
Of
Kyoto University
In Candidacy for the Degree of Doctor of Science

RYU, Heungjin

2017

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To my parents,

나의 부모님께,

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Abstract

My PhD research focused on the mechanism and socio-sexual functions of sexual swelling in wild female bonobos. Bonobo sexual swelling is prolonged compared that of the chimpanzee. Though bonobos sometimes have very long periods of maximal swelling that continue for over one month, the difference in the average length of maximal swelling phase between the two species was only 2.3 days. It is therefore questionable whether prolonged swelling periods have much of a concealing effect on ovulation. It is also likely that the lower predictability of ovulation from the onset of the maximal swelling phase might be due to inter-intra individual variations in the length of maximal swelling phase depending upon each individual's reproductive history as discussed in Chapter 2.

The physiology of maximal swelling in bonobos was not very different from chimpanzees and other Old World monkeys. Although the exact criterion of E1C concentration cannot be determined in Chapter 2, I confirmed that a rise in urinary E1C coincided with the onset of the maximal swelling phase. I also confirmed that a constant increase of PdG, probably from the corpus luteum, above the baseline after ovulation coincided with detumescence of the maximal swelling. Lactation and early loss of pregnancy probably influence the considerable variation in length of the maximal swelling phase within an individual and between individuals. As reported in Chapter 2, female bonobos resume maximal swelling cycles on an average of 240.5 days after parturition, although females normally do not get pregnant until after 3 years. This early resumption of sexual swelling seems to be a unique feature of female bonobo sexual swellings and might perform some regulative function in male mating competition by allowing more mating opportunities to males.

Lower predictability of ovulation from the onset of maximal swelling might not be a big challenge for males, in that males may have simple rules for allocating their mating efforts

towards certain females, as concluded in Chapter 3. They might decide to follow the female with an older infant, if the female has developed maximal swelling, and increase or maintain mating efforts on the next day if the maximal swelling is continued. It is also possible that males have the ability to discriminate ovulation more precisely using the subtle changes in color or size of swelling as well as other behavioral or olfactory cues. To test this possibility, we need to take more precise measurements of sexual swelling using digital photography.

The sexual swelling of female bonobos could also play an important role in female-female bonding, as addressed in Chapter 4. Females with maximal swelling increase attractiveness to both males and females and this might allow those females to get some benefit from group living. Though I do not propose the evolutionary mechanism in this study, it is possible that signals from sexual swellings influence other females' attitude to the females with maximal swelling. Given that the sexual swelling is a very important body part and the cost of signaling using that vulnerable body part is high, the sexual swelling can serve as an honest signal to evaluate another's motivation or the relationship between two individuals.

Lastly, although the early resumption of sexual swelling might have a regulative role in male mating competition to some extent, this study showed that male mating competition prevails in bonobos and male rank is an important factor in reproductive success, as found in chimpanzees. As shown in Chapter 3, male bonobos allocate their mating efforts based on variations in the sexual swelling of females. If male bonobos can precisely extrapolate ovulation probability, as males can in the other species that exhibit sexual swelling, it would be difficult to maintain the argument that milder male–male competition for reproduction in bonobos is explained by a poor predictability of ovulation. The high social status (or balanced power holding) of female bonobos can influence male mating competition, as the high social status of females might allow female choice to exert influence on males' reproductive

success. If that is the case, then less restriction on copulation opportunities for lower ranking males may also be due to female choice. Therefore, in future research, it will be important to investigate how female bonobos can achieve high social status in their society, as well as the socio-regulative role of prolonged receptivity of female bonobos. This will allow us to evaluate whether the hyper-sexuality of bonobos results in peaceful bonobo society, or whether hyper-sexuality itself is a result of more balanced power holding between sexes.

Acknowledgements

First of all, I would like to thank my mother, father, and brother for supporting me studying in Japan for 7 years. Without their support and understanding, I could have not finished my PhD study.

I would like to thank Dr. Furuichi as my main advisor. He has always helped me by encouraging the study. His contributions were essential for the whole thesis.

I thank Dr. Hill for his supervising and contribution to chapters 3 and 4. I also thank Dr. Hashimoto for her supervising and contribution for Chapter 2 of this dissertation. I thank Ms. Mouri and Dr. Shimizu for their contribution to the hormonal assay. I also thank Dr. Hosaka, Dr. Miyabe, and Dr. Bercovitch for their great contributions and for giving me comments on the ongoing writing of this manuscript.

I would like to thank our research assistants at Wamba, especially Mr. Bafutsa, Mr. Bafaluka, Mr. Etenyi, Mr. Batsindelia, Mr. Isoluka, and Mr. Bafike for their great help. I thank Dr. Graham and Dr. MacIntosh for great help in the revision of English and support. I also thank the members of the Department of Ecology and Social Behavior and CICASP at the Primate Research Institute of Kyoto University, in particular Dr. Sakamaki, Dr. Tsuji, Dr. Nishikawa, Dr. Tokuyama, Dr. Yumoto, Dr. Regail, Dr. Kinoshita, Dr. Huffman Dr. Takemoto, Dr. Sawada, Dr. Matsuzawa, Dr. Adachi, Ms. Hirose, Ms. Yamamoto, Mr. Toda, Ms. Renata, Ms. Yanai, Ms. Kim, and Ms. Kawaguchi for comments and help.

For the last 7 years, my study in Japan was supported by three international scholarship foundations [Seiwa international scholarship foundation (2011-2012), Mitsubishi corporation international scholarship foundation (2013-2015), Iwatani Naoji international scholarship foundation (2016)] and two of my great friends Park, Hyungjin and Jung, Jaeho. Their contribution was indispensable for this dissertation. If they did not support me, I could not have continued studying in Japan.

This study was supported by the Environment Research and Technology Development Fund (D-1007 to Furuichi), Japan Society for the Promotion of Science (JSPS) Grants-in-aid for Scientific Research (22255007 to Furuichi), JSPS Grants-in-aid for Scientific Research (25304019 to Hashimoto), the JSPS Grants-in-aid for Scientific Research (25257409 to Ihobe), the JSPS Asia-Africa Science Platform Program (2009-2011, 2012-2014 to Furuichi), JSPS ITP-HOPE Project (ITP-23-012 to Ryu, Heungjin), and JSPS AS-HOPE Project (AS-24-007 to Ryu, Heungjin). I thank the Ministry of Scientific and Technological Research of the D.R. Congo for research permissions (No MIN.RS/SG/013/2011, MIN.ESURS/SG/010/2012, MIN.ESURS/SG-RST/13/2013, MIN.ESURS/SG-RST/007/2014, MIN.ESURS/SG-RST/026/2014) and the Research Center of Ecology and Forestry for helping us and maintaining Luo Scientific Reserve.

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1. Chapter 1

General Introduction

1.1 Sexual swelling in primates in general

Conspicuous primate sexual swellings, namely the size and color of females' genitalia, have captured the attention of primatology since first documented (Darwin 1876; Pocock 1925). Later it was also found that sexual swellings, regardless of visibility, are widespread in various primate taxa (Chapter 4; Dixson 2012). However, the term "sexual swelling" has been mostly used to describe the sexual swelling of females in several Old World monkeys (e.g species in genus *Papio* and several species in genus *Macaca*) and the two *Pan* species (for more details please see van Schaik et al. 1999; Nunn 1999). The size and color of the sexual swelling found in these species are exaggerated and change in relation to menstrual cycle. To distinguish this very conspicuous sexual swelling in size and color, Nunn (1999) proposed to call it an "exaggerated sexual swelling". However, I will mostly use the term sexual swelling instead of exaggerated sexual swelling to save space and to avoid confusion from the conventional way that researchers use sexual swelling (to mean the periodic perineal skin swelling), to describe the sex skin swelling of female bonobos. Therefore, if there is no special note, the term sexual swelling is the synonym of exaggerated sexual swelling, found only in several Old World monkeys and the two *Pan* species, for the rest of this thesis.

There are many hypotheses, which explain the socio-sexual functions of the sexual swelling in Old World monkeys and the two *Pan* species (Chapter 4). Since the sexual swelling is found in many different species and evolved at least 3 times independently (van Schaik et al. 1999; Nunn 1999), it is not easy to find a model for all proposed functions of the sexual swelling. However, the finding that most species that exhibit sexual swelling have multi-male and multi-female social system (van Schaik et al. 1999), seems to be key to understanding the functions of sexual swelling. The correlation between the existence of

sexual swelling and multi-male and multi-female social systems suggests that females' sexual swelling allows females to have some control on males' reproductive outcomes, as well as their own, in multi-male and multi-female societies (Clutton-Brock and Harvey 1976; Nunn 1999; Street et al. 2016). More details of the proposed hypotheses and discussion are reviewed in Chapter 4.

The graded-signal hypothesis, assumes that sexual swelling (exaggerated) delivers a graded-signal which indicates probability of ovulation to males through the changes in swelling size in relation to serum estrogen and progesterone concentration (Nunn 1999). It has been demonstrated that an increase in estrogen is responsible for the increase in size of sexual swelling, and an increase in progesterone after ovulation results in detumescence (Gillman 1940; Kato et al. 1980; Ozasa and Gould 1982). This physiological mechanism therefore allows males to rely on the sexual swelling as a graded-signal as a reliable indicator of ovulation (Nunn 1999; Street et al. 2016).

This graded-signal hypothesis has been the most comprehensive among the several hypotheses and has most successfully explained the function of sexual swelling in different species. The graded-signal hypothesis has also been supported by quantitative data from field and captive studies (Emery and Whitten 2003; Deschner et al. 2004; Brauch et al. 2007; Daspre et al. 2009; Higham et al. 2012). However, this hypothesis does not provide any models to deal with inter-species variation in the reliability of the signal, i.e. how reliably the sexual signal conveys information on the probability of ovulation. In other words, it does not provide any testable predictions regarding how much inter-species variation and inter-individual variation within a species exists and, if any, the reasons these variations exist. One of humans' two closest relatives, bonobos (*Pan paniscus*) provide a valuable opportunity to examine the topic, since they are thought to be the species with the longest period of maximal sexual swelling and the most unreliable signal to predict ovulation (Street et al. 2016).

1.2 Sexual swelling in bonobos and chimpanzees

As mentioned above, the sexual swellings of bonobos and chimpanzees are exaggerated in size and color (see also Figure 1-1). The sexual swelling of bonobos and chimpanzees changes depending upon serum estrogen and progesterone concentration. In chimpanzees, the average period of maximal swelling seems to be similar across several field sites including Eastern and Western populations (Table 1-1). A study which consisted of 46 individuals from Gombe, Budongo and Kibale (Eastern chimpanzees), showed that the average of maximal swelling periods was 12.7 days (Thompson 2005). Two studies from Mahale chimpanzees – also Eastern chimpanzees – showed a similar tendency (12.5 days; Hasegawa and Hiraiwa-Hasegawa 1983, 10.9 days; Matsumoto-Oda and Oda 1998). This tendency is consistent with the Western chimpanzee population in Taï (10.9 days; Deschner et al. 2003) and also in captive conditions (10.4 days; Yerkes and Elder 1936). However, it seems that there is greater variation in the period of maximal swelling phase in bonobos (Table 1-1, see also Figure 5-3).

It has been suggested that sexuality of bonobos regulates the tension from group living (de Waal 1990; Furuichi 1992; Parish 1994; Hohmann and Fruth 2000). The prolonged receptive periods (also called prolonged estrus), therefore, were assumed to allow female bonobos to engage in sexual interactions more frequently and thus lower male-male mating competition and contribute towards the peaceful bonobo society (Furuichi 2011). Furuichi (2011) showed that the more frequent maximal swelling phases of female bonobos, compared with that of chimpanzees – female bonobos exhibit maximal swelling within 1 year of parturition and continue their swelling cycles for around 3 years, until the next pregnancy –, results in an increased number of receptive females (“estrous females” in his paper) in a bonobo unit-group (or community). Together with an assumption that the maximal swelling of bonobos plays an important role in concealing ovulation, it seems reasonable to assume

that an increased number of receptive females reduces male-male competition over mating. Furthermore, Furuichi (2011) suggested that if males do not need to compete with each other over access to females, then they do not need to keep aggressive characteristics. A reduction in male aggressiveness over generations may be the most plausible scenario of the evolution of peaceful bonobo society.

Together with the earlier resumption of maximal swelling cycle of female bonobos (Furuichi and Hashimoto 2002), this hypothesis seems to be supported by the findings that the probability of ovulation and the maximal swelling periods is loosely related in bonobos (Heistermann et al. 1996; Reichert et al. 2002; Douglas et al. 2016). However, the relationship between maximal swelling and ovulation is not fully understood yet. Among all available studies, the two studies which used the most individuals and cycles (23 cycles from 8 females in Reichert et al. 2002 and 26 cycles of 9 females in Douglas et al. 2016) drew a slightly different conclusion. Reichert et al (2002) found that ovulations took place in the latter half of the maximal swelling phase, whereas Douglas et al (2016) found that occurrences of ovulation are evenly distributed throughout the maximal swelling phase. Moreover, the most recent review paper that compared sexual swelling in several primates species (Street et al. 2016), concluded that sexual swelling in the bonobo is indeed a reliable indicator of ovulation. However, that review did not include the most recent study published by Douglas and colleagues in 2016.

If the sexual swelling of bonobos is not a reliable signal of ovulation, it is questionable how the physiological mechanism resulting in the unreliability of signaling of ovulation has evolved only in bonobos. When serum estrogen concentration gets higher, the sexual swelling gets bigger and reaches its maximal size with the peak of estrogen concentration. After the peak of estrogen concentration, if ovulation takes place, serum progesterone concentration increases and the size of sexual swelling decreases (detumescence). Though there is variation

in the number of days to reach detumescence after ovulation (mostly within a week, however), secretion of progesterone from the corpus luteum generally induces the detumescence of sexual swelling. No physiological mechanism has been proposed to explain changes in size of sexual swelling, in any of the species that have sexual swellings, other than the regulatory mechanism of estrogen and progesterone. It is also only bonobos, among all species with sexual swellings, that the exceptional relationships – maximal swelling takes place after ovulation or before ovulation – between maximal swelling and unmatched ovulation has been reported.

1.3 Backgrounds and definitions

In many non-primate mammal species the term "*Estrus: the special period of sexual desire of the female coincided with ovulation*" is meaningful since females are sexually active only in a short period near to ovulation (Heape 1900; Dixson 2012). However, in many primate species, particularly in anthropoids, the term estrus has been challenged (Beach 1976; Dixson 2012). Dixson even stated in his book – Primate Sexuality 2012, page 478 – that '*Among the anthropoids, the concept of oestrus is of no value*', largely because of the extended sexual behavior of females in many primate species outside of ovulation (prolonged receptivity). Since the term estrus is mainly measured by receptivity of female mammals and the receptive period is usually limited near to ovulation, the term estrus is also considered to include fertile periods. However, in many primate species, sexual behavior is not only limited around ovulation. In particular, bonobos, often recognized as the most sexually active animals, are famous for their non-conceptive mating (de Waal 1995; Furuichi et al. 2014). I therefore specify a female with maximal swelling or a female in the fertile phase (the 4 day fertile window; also called peri-ovulatory periods by Deschner and colleagues in Deschner et al. 2003), instead of using the term "estrus female" throughout this dissertation.

In this dissertation, I chose to frame the sexual cycle in terms of prolonged periods of maximal swelling phase, rather than "estrus". The prolonged periods of sexual swelling phase combines two components. One is that female bonobos exhibit (or develop) maximal tumescence of sexual swelling more frequently than do female chimpanzees, from a successful parturition to the next parturition (between two successful births). This can be indirectly interpreted as prolonged periods of maximal swelling. The other is that the duration of maximal tumescence of the sexual swelling of female bonobos may also be prolonged compared to that of female chimpanzees (Table 1-1, see also Table 5-1, Figure 5-3 in Chapter 5), although published data is currently inconclusive. Nulliparous female bonobos exhibit very long periods of maximal swelling (Furuichi 1987; Ryu et al. 2015) and so do females in early lactation periods (from 1 year to 2 years after giving birth; Chapter 2). Both factors contributing to the possible prolonged duration of the maximal swelling phase are included in the term "prolonged maximal swelling" in this dissertation.

The operational sex ratio might be an important factor for understanding male-male competition (Mitani et al. 1996; Furuichi 2011). However, considering the similarity of non-seasonal breeding systems, inter-birth interval (Furuichi and Hashimoto 2002), and adult male-female sex ratio (1:2 to 4 in chimpanzees, around 1:1.2 in bonobos, more details see Stumpf 2011) between chimpanzees and bonobos, it is likely that male-male competition over reproduction (not the mating competition) is not dissimilar between these two species. In fact, male chimpanzees would be predicted to have even lower competition given the higher adult sex ratio. Although it is possible that group size and the absolute number of males in a unit-group influence the degree of male-male competition, this is beyond the scope of this dissertation.

One of the biggest methodological challenges was preserving urine samples. Since sufficient electrical supply was missing at Wamba, and transportation of large quantities of

liquid nitrogen was prohibitively difficult and expensive, it was not possible to freeze the urine samples. In addition, since transferring frozen samples from DR Congo to Japan costs several thousand dollars, a solution was needed. I found that using filter papers to collect the urine samples seemed to be a suitable solution for this study.

Collecting urine using a filter paper was not complicated. After a female bonobo urinated on a branch and moved away, I (or one of the researchers or assistants) moved to where the female bonobo had urinated. Then I put the tip of a filter paper (around 1/4 of a round filter paper) into the urine drops on the leaves. When the filter paper absorbed urine to more than a half of the paper, I stopped absorbing and withdrew the paper. After returning to the camp, I dried the filter paper in a dry box with silica gel. It usually took 2 days until the filter paper was completely dried, but I left the paper in the box for at least one week as a precaution.

Urine sample collection with filter papers was validated in studies investigating urinary E1C and PdG from captive long-tailed macaques (Shideler et al. 1995) and wild orangutans (Knott 2005). These two studies confirmed that the methodology was reliable by showing that the recovery rate of hormonal levels from filter paper extracts were highly correlated with the hormonal level from original urine samples (over 80%). In addition, for the last few years, Ms. Mouri and Dr. Shimizu have been developing a simpler method based on the two previous papers (Mouri and Shimizu in prep). Ms. Mouri and Dr. Shimizu used deionized water to extract the urine from the filter paper. It was thought that deionized water (or distilled water) was not a good solvent to extract urine from filter paper (Knott 2005). However, Ms. Mouri and Dr. Shimizu found that by using mechanical shaking during the extraction process, the extraction efficiency improved to the same reliable levels showed in the previous two papers (Mouri and Shimizu 2014). My study is the first study using Mouri and Shimizu's methods on wild bonobos.

1.4 Organization of the dissertation

In this dissertation, I include three empirical studies on wild bonobos. In Chapter 2, I present data on the periods of the maximal swelling phase and its associations with sexual hormones metabolites found in urine – estrogen conjugate (E1C) and pregnanediol 3-glucuronide (PdG). In Chapter 3, I describe how male bonobos respond to a female with maximal swelling and discuss how males deal with the different reproductive quality and probability of ovulation of female bonobos over time, depending on reproductive history and menstrual cycle. In Chapter 4, I propose an additional function of female bonobo sexual swelling; that it also functions to modulate female-female affiliative social interactions and thus contributes to female-female association. Chapter 1 is the general introduction of the study and Chapter 5 is the summarizing discussion of the dissertation.

1.5 Aims of the research

In this thesis, I first tried to examine the relationship between sexual swelling and the probability of ovulation (Chapter 2). I collected urine samples and recorded changes in sexual swelling of female bonobos (Figure1-1) in a wild bonobo group called E1 (Figure 1-2) at Wamba in the Luo Scientific Reserve, DR Congo. This study would not have been feasible in captivity since most captive populations are under contraceptive control. The study group is called E1 and was originally a part of E group, which had been fully identified and habituated by 1976. When it was habituated, E group probably had two sub-units within the group. Later, E group split into two groups (E1 and E2) and E1 group became an independent group by 1983 (Furuichi 1987). There were interruptions to field studies from 1996 to 2002 because of civil wars in DR Congo. This group was under artificial provisioning until 1996. However, after researchers resumed the study in 2002, no artificial provisioning was conducted at Wamba (Furuichi et al. 2012).

The other aim of this thesis was to test the hypothesis that male–male competition over mating is reduced in bonobos because there are more available partners, resulting from the prolonged periods of maximal swelling. This hypothesis relies on the assumption that bonobo males are not good at discriminating the difference in fertility (probability of ovulation) of females or that they do not care (or care less) about the fertility of females (probability of conception) if they can copulate. This assumption has not been tested yet. The main goal of Chapter 3, therefore, was to test this assumption.

Sexual activity of female simians (Old World monkeys and apes) is not strictly restricted within their short ovulatory window (Dixson 2012). Therefore, it is unlikely that outcompeting other males over copulation per se is directly interpretable as outcompeting other males over reproduction. If male bonobos cannot successfully extrapolate the probability of ovulation or the reproductive quality of females, then an increase in available partners for copulation can lowers the male-male competition over mating. In this case, female mate choice and sperm competition among males might also strongly influence the reproductive success of males. However, if males cannot discriminate the ovulation probability, my results would support the female estrous sex-ratio hypothesis proposed by Furuichi (Furuichi 2011) to explain the peaceful bonobo society. In Chapter 3, I examined how males differentiate their behavioral response toward females with different probability of ovulation and infant age.

The duration of the maximal swelling phase of bonobos might have been selected to have greater inter-cycle variation and resume during nursing periods (Furuichi and Hashimoto 2002; Chapter 2). It is also possible that it could be prolonged compared to that of chimpanzees (Table 1-1, and see also Table 5-1, Figure 5-3 in Chapter 5). One possible mechanism of the greater variation and prolonged duration of the maximal swelling phase of female bonobos may be increased sensitivity of sexual skin to estrogen (Tobias Deschner,

personal communication). It might be case that the density of estrogen receptors of the sexual skin is higher in bonobos or that the cells in sexual skin could have hyper-activity in response to an increase in serum estrogen. In any case, it seems that one reason for the variation and prolonged maximal swelling phase of female bonobos is due to the follicular phase (after menstruation to the next ovulation), of which the length can vary between cycles and individuals. High variation in the duration of the follicular phase was noted in captive and wild bonobos (Heistermann et al. 1996; Douglas et al. 2016). Together with the assumption that sensitivity of bonobo sexual skin to estrogen has increased, it is likely that the length of the follicular phase is an important source of the greater inter-intra individual variations in the length of the maximal swelling phase, as well as prolonged maximal swelling phase.

If sexual swelling of female bonobos has indeed been selected to be more estrogen-sensitive and thus more likely to reach its maximal size more often and for longer, then why was it selected like that? It is known that the copulation rate of female bonobos increases during the maximal swelling phase (Furuichi 1987; Ryu et al. 2015). However, facilitating copulation might not be the only reason for the prolonged maximal swelling phase. In Chapter 4, I suggested that female bonobo sexual swelling also mediates female-female affiliative interactions. Given that genito-genital rubbing is found almost uniquely in bonobos (Kuroda 1980), rarely in other species such as mountain gorillas; (Grueter and Stoinski 2016) and that it is most commonly observed between two females with maximal swelling, prolonged periods of maximal swelling might give females the extra benefit of forming female-female social bonds. Female–female bonding is known to be crucial to group living of female bonobos in the wild (Hohmann and Fruth 2000; Tokuyama and Furuichi 2016) and captivity (Parish 1994). Therefore, I would propose that bonobos may have evolved a more prolonged maximal swelling compared with that of chimpanzees, to cope with males' mating

strategies and needs for female–female bonding, rather than to consider it as an artifact of categorical measurement bias of sexual swelling by researchers in different field sites.

Table 1-1. Periods of maximal swelling phase in chimpanzees and bonobos

Species	Condition	No. cycles	No. id	avg	sd *se	Site	Study
Chimpanzee	W	41	46	12.7	-	Gombe, Kibale, Budongo	(Thompson 2005)
Chimpanzee	W	33	12	10.9	3.2	Taï	(Deschner et al. 2003)
Chimpanzee	W	27	28	12.5	-	Mahale 1	(Hasegawa and Hiraiwa-Hasegawa 1983)
Chimpanzee	W	37	5	10.9	-	Mahale 2	(Matsumoto-Oda and Oda 1998)
Chimpanzee	C	53	13	11.9	4.4	Norman, OK	(Wallis 1992)
Chimpanzee	W	37	6	9.6	-	Gombe	(Tutin and McGinnis 1981)
Chimpanzee	C	158	10	10.4	-	Yerkes	(Yerkes and Elder 1936)
Bonobo	C	9	4	11.5	2.7*	Planckendael	(Heistermann et al. 1996)
Bonobo	C	23	8	16.0	6.8	Cologne and 3 others	(Reichert et al. 2002)
Bonobo	W	8	6	10.6	6.8	Luikotale	(Douglas et al. 2016)
Bonobo	W	9	3	14.6	7.4	Wamba 1	(Furuichi 1987)
Bonobo	W	9	-	12.9	9.3	Wamba 2	(Takahata et al. 1996)
Bonobo	C	57	4	13.4	0.7*	Apenheul	(Paoli et al. 2006)
Bonobo	C	6	1	15.3	-	Yerkes	(Savage-Rumbaugh and Wilkerson 1978)

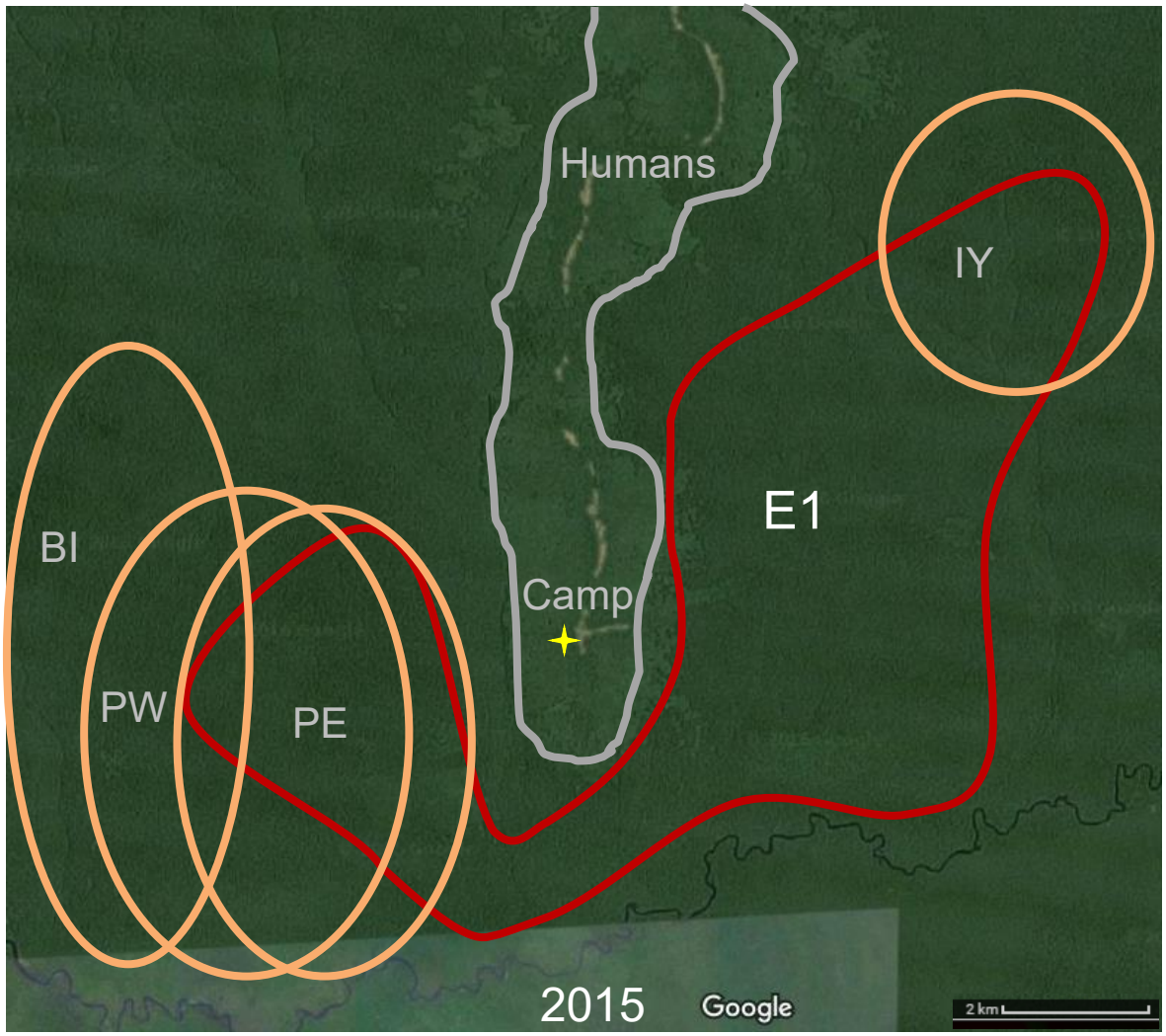
No. cycles: the number of cycles, No. id: the number of individuals, avg: average, sd: standard deviation, *se: standard error, W: wild, C: captivity, -: not available

Figure 1-1. Morphological changes in sexual swelling within a swelling cycle of one individual named Fk

a) Non swelling; swelling score 1
b) Intermediate swelling; swelling score 2
c) Maximal swelling; swelling score 3

Figure 1-2. Study population and approximation of boundary of group range

E1 group is the study group of this study.



2. Chapter 2

Post-parturition resumption of ovarian cycles and reproduction by female bonobos

2.1 Abstract

Although chimpanzees and bonobos are closely related, they exhibit considerable differences in numerous social aspects. Especially, the most distinctive feature of the bonobo society is the peaceful nature, including the affinitive intergroup relationship, and no records of infanticide. Female sexual receptivity may contribute to the peaceful nature of the bonobo society. Previous studies have found that female bonobos show maximal swelling during pregnancy or postpartum amenorrhea. However, detailed information on the reproductive physiology of female bonobos during the postpartum period is very limited. In this study, we investigated the dynamics of urinary metabolites of steroid sex hormones (urinary estrone conjugate; E1C and pregnanediol glucuronide; PdG) and changes in reproductive parameters (length and interval of maximal swelling phase, MSP) in relation to time after parturition. The length of each MSP and the proportion of days during which a female bonobo showed maximal swelling increased with an increasing number of days after parturition. The average E1C levels four days prior to ovulation also increased as the time after parturition increased. The E1C level of cycles that led to successful pregnancies was significantly higher than that of non-conception and early loss cycles. In addition, high E1C levels were confirmed only in females evaluated approximately 3.5 years after parturition in this study. The examination of all MSP with confirmed or unconfirmed ovulation revealed that peak E1C levels during the MSP increased with increasing days after parturition. In addition, the E1C levels of the MSP that led to a successful pregnancy were higher than those of the other conditions. These results indicate that female bonobos appeared to need approximately 3 years to recover

physically after lightening the energy burden of lactation, similar to female chimpanzees. However, female bonobos resume maximal swelling much earlier than female chimpanzees do. Female bonobos may resume maximal swelling even at lower levels of estrogen for social purposes such as reduction of male sexual competition, the high social status of females, and development of close female associations even when they are not yet ready for conception.

2.2 Introduction

Bonobos and chimpanzees are almost genetically identical (99.6% in the genome; Prüfer et al. 2012). However, they exhibit numerous differences in numerous social aspects. Especially, the most distinctive feature of the bonobo society is the peaceful nature of the species (Kano 1992; Furuichi 2011). Although numerous cases of intraspecific killings have been reported in chimpanzees (Wilson et al. 2014), no similar cases including infanticides have been reported in bonobos. Moreover, while intergroup relationships are very antagonistic among chimpanzees (Wilson et al. 2014), various affinitive interactions such as genito-genital rubbing, copulation, and peering have been observed between members of different unit-groups during intergroup encounters in bonobos (Idani 1991). The peaceful nature of bonobos may be attributed to the high social status of females (Furuichi 2011; Clay et al. 2016).

Previous studies have revealed the aggressiveness of male chimpanzees and their high social dominance over the females. Wilson et al (2014) showed that male chimpanzees were attackers and victims in most of the observed and supposed lethal aggressions. All male chimpanzees dominate the females (Takahata 1990; Takahata 1990), who tend to forage alone with their offspring while female-female relationships are not strong (Goodall 1986; Gilby and Wrangham 2008; Hashimoto and Furuichi 2015). However, recent studies have suggested there may be some variations in the gregariousness of female chimpanzees in the

wild (Boesch and Boesch-Achermann 2000; Lehmann and Boesch 2008; Itoh and Nakamura 2015). In contrast, female bonobos aggregate at the center of mixed-sex parties (Furuichi 1989; Mulavwa et al. 2008). Female bonobos form close social associations with each other, and in particular, immigrant females have affiliative interactions such as genito-genital rubbing and peering with older females (Furuichi 1989; Idani 1991). Dominance between males and females is equal or equivocal, but female bonobos might have predominant access to food (Furuichi 1997).

Furuichi (2011) suggested that the high social status of female bonobos might be attributed to long periods of receptivity caused by the early resumption of sexual swelling cycles and sexual swelling during pregnancy. Female bonobos resume sexual swelling cycling approximately 1 year (Kano 1992; Furuichi and Hashimoto 2002) or 7.0 ± 4.8 months (Douglas et al. 2016) after parturition, which they repeat until their next pregnancy. However, only one case of parturition has been observed during the 3 years before a subsequent pregnancy in bonobos at Wamba and, therefore, the duration of postpartum infertility in bonobos may be approximately 2–3 years. This suggests that the duration of postpartum infertility of bonobos is similar to that of chimpanzees (Fujita 2015). Therefore, sexual swelling and behavior at the resumption of sexual swelling and sexual behavior at the end of postpartum infertility do not appear to lead to successful conception. Furthermore, female bonobos continue showing maximal swelling and copulate for up to 6.5 months (Furuichi and Hashimoto 2002) or 1–6 months (Douglas et al. 2016) after conception although they are obviously infertile. The receptive period of females in these infertile periods is called “pseudo estrus” (Furuichi 2011; Furuichi et al. 2014).

Furuichi (2011) proposed that the long “pseudo estrus” contributes to the increase in the number of receptive females in the group and mitigates inter-male sexual competition in bonobos. Because of the long inter-birth interval, the number of receptive females per male is

very low in chimpanzees, and high-ranking males can monopolize a fertile female. In contrast, there are 1.5–4.4 times as many receptive female bonobos as there are receptive female chimpanzees because of "pseudo estrus," and it may be difficult for high-ranking males to monopolize fertile females in bonobos (Furuichi and Hashimoto 2002). In line with this observation, the copulation frequency of females during maximal swelling phase is lower in bonobos than it is in chimpanzees (Furuichi and Hashimoto 2002).

However, information on the reproductive physiology of female bonobos during the postpartum period is very limited except for a report of a study on bonobos in Luikotale (Douglas et al. 2016). In this present study, we analyzed the hormonal dynamics of female bonobos using non-invasive urine sampling and the patterns of sexual swelling using long-term data over a 9-year period. Furthermore, we sought to examine how female bonobos resume sexual (menstrual cycle and swelling) cycling and identify changes in their physiological condition from parturition to the subsequent conception. Together with the findings from bonobos in Luikotale (Douglas et al. 2016), this present study of bonobos in Wamba is expected to contribute to our understanding of the unique features of sexual swelling and the sexuality of female bonobos as well as their roles in the bonobo society.

2.3 Materials and Methods

Study site and subjects

The field research study of bonobos was conducted at Wamba in the northern sector of the Luo Scientific Reserve, Democratic Republic of Congo (Kano 1992; Furuichi et al. 2012). The research camp is situated in the center of the northern section of the reserve (0°11′07.6″N, 22°37′57.5″E; WGS84). The studies on bonobos began with a unit group at Wamba in 1974 designated as E, which was divided into the El and E2 groups between 1982 and 1983 (Kuroda 1979; Kitamura 1983; Furuichi 1987; Kano 1992). The bonobos were

provisioned with sugar cane during the observation period from 1976 until 1996 (Kano 1992; Hashimoto et al. 1998). Furthermore, field observations were stopped from 1997 to 2002 because of a civil war (Furuichi et al. 2012). The history of E1 and the details of the study site have been described in numerous reports (Furuichi 1989; Kano 1992; Hashimoto et al. 2008; Idani et al. 2008). The annual rainfall is approximately 2800–2900 mm, and although it rains throughout the year, it rains more in the area around October/November and less around January/February (Mulavwa et al. 2008).

I conducted three field observations of the E1 bonobo group from 2013 to 2015 (September 2013 to February 2014, July 2014 to September 2014, and November 2014 to April 2015). The study group (E1) comprised 32–38 individuals, including seven adult and four adolescent males and nine adult and three adolescent immigrant females during the study periods (see the Tables 3-1).

Scoring sexual and maximal swelling phases

The daily state of the anogenital region wrinkling was scored according to a previously established method (Furuichi 1987), and assigned one of the following three categories as described by (Furuichi 1987): non-swelling status (swelling 1), the sexual skin is highly wrinkled and sways when the animal is walking; intermediate swelling status (swelling 2), the sexual skin is turgid, but small wrinkles are visible on its surface; and maximal swelling status (swelling 3), the sexual skin appears firm and lustrous without wrinkles (see Figure 1-1 in Chapter 1). When the sexual skin reached its maximal swelling status, this was defined as the maximal swelling phase (MSP). To avoid observation bias, at least one researcher and two research assistants recorded the observations independently, and the scores were subsequently determined after discussions at the daily evening meetings.

The duration of the MSP was calculated from the first to the last day of swelling 3. In several cycles, the swelling scores temporarily dropped to stage 2 and then return to swelling 3 without dropping to stage 1 during the MSP. Moreover, observation gaps occurred during the MSP. In those cases, even if there was a temporary drop to swelling 2 or an observation gap occurred, all the days from the onset of swelling to the last day of swelling 3 were defined as one MSP. The maximum length of a temporary drop was 6 days and an observation gap was 6 and 4 days, respectively. Only cycles that did not have a sample gap greater than 1 day at the onset or end of the MSP were used to analyze variations in the duration of the MSP. This definition was almost compatible with that used by Douglas et al. (2016).

Long-term data

Researchers and research assistants have been recording all births by bonobos of the E1 group from the beginning of the field observation in 1974, although no records were maintained during the absence of researchers from 1997 to 2002. We calculated the inter-birth interval using this long-term data. Moreover, the daily sexual swelling status of the females observed by the researchers or research assistants been recorded since September 2007. We used data from September 2007 to August 2016 to calculate the number of days before the parturition during which the bonobos exhibited maximal swelling and the number of days after the parturition when female bonobos resumed sexual cycling.

Urine sample collection

The author, one of the researchers, or a research assistant, collected urine samples from the nine parous female bonobos that had dropped on the leaves of terrestrial plants or small trees using filter papers (Whatman #1 Ø 5.5-cm). The urine samples were collected after the

bonobos had left the area to maintain a distance of at least 5-m distance between the collector and the bonobos. Urine that was contaminated with feces or the urine of other subjects was not collected. At the base camp, the filter papers with the urine samples were stored in a dry box containing 500 g silica gel for 1 week although it was usually completely dry within 2 days in the dry box. After 1 week, the filter papers were kept individually in plastic zipper bags, which were stored in the dark at the room temperature (20 to 28 ℃) for less than 6 months. The dried samples were delivered to Japan where they were kept in a freezer at -20°C. In total, 660 urine samples were used for all the analyses reported in this chapter.

Hormonal measurement and defining fertile phase

The urine was extracted from the filter papers using deionized water with mechanical shaking using an unpublished method developed Mouri and Shimizu (Mouri and Shimizu 2014). Briefly, we placed each filter paper in an extraction tube with deionized water, which was horizontally shaken using a shaker (TAITEC, Japan) to elute the urinary content into the solution. The estrone (E1C), pregnanediol glucuronide (PdG), and creatinine concentrations of the filter paper urine extracts (stored for 1 year at room temperature) were very highly correlated with the concentrations of the original urine samples (Mouri and Shimizu 2014).

First, the creatinine concentration of the urine extracts of the filter papers was measured using Jaffe reaction methods (Taussky and Kurzmann 1954). Then urinary E1C and PdG concentrations were determined using enzyme immunoassays (EIAs) as described previously in chimpanzees (Shimizu et al. 2003a) and bonobos (Heistermann et al. 1996; Shimizu et al. 2003b; Shimizu 2005). The EIA protocols were validated by Mouri and Shimizu (2014). Then, the urinary E1C and PdG concentrations were indexed by dividing their original concentrations with that of creatinine.

In addition, we used polyclonal antibodies (Cosmo Bio, Japan) raised against estrone-3-glucuronide bovine serum albumin (BSA) and pregnanediol-3-glucuronide BSA, and horseradish peroxide-conjugated steroid derivatives (Cosmo Bio, Japan) for the EIAs. The sensitivities, parallelisms, and recovery tests of the assays were performed using methods described by Mouri and Shimizu (2014). The sensitivities were 6.6 and 2.1 pg/mL for E1C and PdG, respectively. The parallelism was confirmed using constant dilutions of samples from three subjects while the recovery tests were performed by adding E1C and PdG standards to samples from three subjects. ANCOVA tests with the different dilutions from three different bonobo urine samples (N = 3x4) confirmed that there was no serious violation of parallelism between the diluted samples and the standards (*P* values ranges 0.155 to 0.337 for E1C and 0.255 to 0.730 for PdG). The recovery test by adding fixed amount of standard solutions to three different samples (N = 3x4) showed high regression coefficient values (E1C: $Y = 0.99X + 0.01$, $r^2 = 0.985$, PdG: $Y = 0.99X - 2.5$, $r^2 = 0.967$), which suggests that E1C and PdG were successfully recovered in assays. Further details of the methods, parallelism, and recovery tests are available in the report by Mouri and Shimizu (2014) and Mouri and Shimizu (unpublished data).

Cross-reactivity information of the antibodies against PdG and E1C was provided by the manufacturer (Cosmo Bio, Japan). The PdG antibody cross-reacted 100, 16.0, 2.3, and <1% with pregnanediol, 20α-OH-progesterone, progesterone, and other steroids, respectively. The E1C antibody cross-reacted 100, 5.5, 2.5, 1.2, and <1% with estrone-glucuronide, estrone, pregnenolone, testosterone, and other steroids, respectively. Inter plate CV for E1C was 10.79 and 15.83 for PdG. Intra sample CV was 5.66 for E1C and 6.66 for PdG.

The timing of ovulation was calculated from the urinary PdG excretion profiles using methods described in previous studies (Heistermann et al. 2001; Deschner et al. 2003; Douglas et al. 2016). The ovulation date was estimated based on a sustained urinary PdG

increase above the baseline, which was defined as the mean urinary PdG concentrations of the preceding 10 days. A sustained rise in the PdG levels above two standard deviations from the baseline (threshold) was used to detect ovulation. When fewer than three samples were collected within 10 days, they were not used to determine the ovulation cycle. Furthermore, when the average PdG concentration of the 10 days succeeding ovulation was not higher than that of the threshold (mean + 2 standard deviations, SD), we did not consider the rise in PdG above the threshold over 1 or 2 days to have been caused by ovulation. The reason I used the average of 10 succeeding days was that the average length of the luteal phase was 9.5 ± 1.2 days in a previous study on wild bonobos (Douglas et al. 2016). The fertile phase (fertile windows or peri-ovulatory period, POP) was defined as the 3 preceding days from ovulation and the day of ovulation (4 days in total) based on a previous study in chimpanzees (Deschner et al. 2003).

Data analysis

We used the R 3.3.2 (R Core Team 2016a) with RStudio 1.0.44 (RStudio Team 2016) for the generalized linear mixed models (GLMMs) analyses. We used lmer and glmer of lme4 package (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2016) to fit our models to the data and examine the p-values. The response variables were the duration from the last MSP to parturition, duration from parturition to earliest MSP, mean E1C concentration in each peri-ovulatory period, mean of three highest concentrations of E1C for each MSP, interval between MSP, length of MSP, proportion of MSP to all observed days, and mean E1C concentration of sampled days in each swelling status. The predictor variables were the days from parturition and sex, mother's age, and whether the MSP led to a successful pregnancy, as well as the mother's reproductive status (during pregnancy, early lactation, or late lactation) or status of sexual swelling or both, depending on the models for each response

variables (Table 2-1, GLMM 1-a to 1-i). All models included the mother's ID as a random factor. Only one response variable (length of maximal swelling period) was square root transformed (to make it normal distribution) before the analysis because it was skewed to a shorter length (GLMM 1-b, 1-c). We used glmer with Gamma family (using the log-link function) to test the effect of the sexual swelling status and days from parturition on the concentration of E1C (GLMM 1-d). Furthermore, glmer with Binomial family was used to test the effect of years from parturition on the proportion of MSP to observations days (GLMM 1-g), and lmer with a normal distribution was used for all other models. In models with multiple predictors, we examined the multicollinearity between predictors using variance inflation factors (VIFs) and confirmed that there was no serious concern (the maximum VIF value of all the models was 1.29). In all linear mixed models, we confirmed that there was no serious skew or extreme data points, which can violate the models (Cook's distance was less than <1 in all models) or over-variance using the Shapiro test and visual inspection of residuals of the fitted models. The differences were considered statistically significant at $p = 0.05$.

2.4 Results

Inter-birth interval

We calculated the inter-birth interval after each surviving offspring (IBI) for bonobos of E1 group. The mean IBI from 1974 to 2016 was 58.0 ± 10.7 months (range: 43–85, N = 24). We provided sugar cane to the bonobos before 2002 and stopped the supply in 2002; therefore, we calculated the IBI for the period before and after 2002. There was no significant difference in the IBI before (56.2 ± 11.0; range, 43–72; n = 11) and after 2002 (59.5 ± 10.6; (range, 50–85; n = 13; Man-Whitney U test, n1 = 11, n2 = 13; U = 60; Z = 0.668; p = 0.505).

Hormone profile

The analysis of the samples collected from the nine female subjects revealed the timing of ovulation in 14 cycles of five females consisted of 11 non-conception and three conception cycles (two resulted in successful births). We created composite profiles of the urinary E1C and PdG, representing the 11 ovulatory cycles of the four females (Figure 2-1). The dynamics of E1C and PdG were similar to those obtained in previous studies of captive and wild bonobos (Reichert et al. 2002; Shimizu 2005; Douglas et al. 2016). The E1C concentration gradually increased during the follicular phase and peaked before PdG increased. The concentration of PdG remained low during the follicular phase and was elevated during the luteal phase. The inter-ovulation interval was 44.3 ± 10.8 (range, 32–52; n = 3) and the average luteal phase from the ovulation day to the first day of menstruation was 10.6 ± 0.6, which was also similar to previously reported findings (Douglas et al. 2016). We also found the occurrence of ovulation when infants were 2 and 2.5 years old. There was one possible case of ovulation by a female (Nv) with a 1.7-year-old infant (20 months) although the ovulation day could not be determined because we lacked the 5-day sampling data.

We monitored the hormonal dynamics of the two conception cycles until birth (Figure 2-2). The concentration of E1C and PdG increased to extremely high levels. The gestation period was 242 and 230 days for the two cycles that were monitored for all periods. These values are similar to the 229-, 234-, and 238-day gestation periods observed in a previous study of captive bonobos (Heistermann et al. 1996).

In one cycle, we determined the timing ovulation and discovered that the high E1C and PdG concentrations persisted for more than 2 weeks (Figure 2-3) and then decreased to normal concentrations thereafter. This was probably a case of early loss, which has been previously reported in humans and chimpanzees (Wilcox et al. 1988; Thompson 2005).

Maximal swelling before and after birthing

Previous studies have found that female bonobos resume maximal swelling in the early stage of postpartum amenorrhea and during pregnancy (Furuichi 1987; Kano 1992; Douglas et al. 2016). We monitored the time until the female bonobos continue maximal swelling before the parturition and the number of days before the female bonobos resumed maximal swelling after parturition using the long-term data from September 2007 to August 2016. The mean duration from the last maximal swelling day to parturition was 152.4 ± 44.5 days (range, 72–222; n = 19), which indicates that female bonobos exhibit maximal swelling within 2.5 months after impregnation when the assumed gestation period was 236 days (average of two gestation periods of 230 and 242 days). The sex of the offspring and age of the mother had no effect on the duration (GLMM 1-e, Table 2-1). The mean duration from parturition to the earliest maximal swelling was 240.5 ± 140.7 days (range, 32–516; n = 15). Furthermore, 40% of the females resumed maximal swelling before half a year and two-thirds of females resumed maximal swelling within 1 year from birthing. The sex of the offspring and age of the mother had no significant effect on the duration (GLMM 1-f, Table 2-1).

Female sexual swelling cycle: Interswelling interval (ISI) and duration of MSP

Similar to previous studies in captivity and the wild (Furuichi 1987; Heistermann et al. 1996; Douglas et al. 2016), the firmness of the sexual skin showed a cyclic fluctuation. The mean menstrual cycle duration was inferred from the ISI, which was defined as the duration from the first day of one MSP to the first day of the next. The mean menstrual cycle duration value was 37.0 ± 15.6 days (range, 11–67; n = 24). The days from parturition had no significant effect on the ISI (GLMM 1-a, Table 2-1).

The duration of the MSP was calculated as the period from the first to the last day of swelling 3. The mean duration of the MSP was 14.0 ± 11.2 days (range, 1–45; n = 36).

Similar to a previous report (Douglas et al. 2016), the duration of MSP increased significantly with increasing days from parturition (Figure 2-4, GLMM 1-b, Table 2-1). However, it is important to note that this model was fitted with the square root of the length of maximal swelling and the variations in the MSP within 2 years of parturition was large whereas the duration of MSP stabilized after 2 years of parturition (Figure 2-4). Classifying the female reproductive status into three categories revealed that pregnancy, early and late nursing periods, and the duration of MSP were significantly longer during the late nursing period than they were during the early nursing period or pregnancy (GLMM 1-c, Table 2-1). The early nursing period was defined as the time from parturition to the average maximal swelling resumption day, which might be the period during which an infant heavily relies on the milk from its mother (the average was 240.5 days after birth in this study). Therefore, the late nursing period was set as 240.5 days after birthing to the next conception. This period might reflect the time when the weaning of an infant starts, and it begins eating solid food while breastfeeding.

E1C concentrations in each swelling phase

The sexual swelling status corresponded to E1C concentrations (Figure 2-5, GLMM 1-d, Table 2-1). The E1C concentration of urine of females in the MSP (swelling 3) was higher than that of females in intermediate swelling (swelling 2). E1C concentrations of swelling 2 were also higher than those of swelling 1. Furthermore, years from parturition showed no significant effect on the E1C concentration.

Timing of ovulation relative to MSPs

Figure 2-6 shows the timing of ovulation relative to the MSP of 14 cycles and only one ovulation cycle occurred close to the end of the MSP (detumescence). Copulation was more

likely observed in the MSP than in other phases (chapter 3), although some copulation was observed out of MSP.

Proportion of MSP to observation days

As mentioned above, maximal swelling resumed an average of 240.5 days after parturitions and the proportion of maximal swelling significantly increased with increasing years from parturition (Figure 2-7, GLMM1-g, Table 2-1). Furthermore, 4 years after parturition, most females showed a maximal swelling for more than 50% of the days (but after 5 years it was one female), suggesting that the females showed MSP for a larger proportion of the days when they reached higher fertility periods.

Difference in E1C concentrations between cycles

We examined the average E1C level during the four days prior to the ovulation day of 14 cycles for which we could determine the days of ovulation (Figure 2-8). The days from parturition had no significant effect on the average E1C level, and the average E1C levels that led to a successful pregnancy were significantly higher than those that resulted in non-conception cycles (GLMM 1-h, Table 2-1). The average E1C levels of the early loss cycles were as low as the levels of the non-conception cycles.

Changes in the three highest mean E1C concentrations of each MSP

To examine changes in E1C concentrations over a wide range of days from parturition, we examined the change in E1C levels in all cycles with or without determining the days of ovulation. In this analysis, we examined peak E1C concentrations of the MSP by calculating the mean of three E1C concentrations in each MSP. We did not include MSPs that had the data of E1C levels for ≤ 4 days (Figure 2-9). In this dataset, we found that the E1C

concentration significantly increased with increasing days from parturition, and the level was significantly higher in the two successful conception cycles than it was in the other 12 cycles (GLMM 1-I, Table 2-1).

2.5 Discussion

Female bonobos show maximal swelling during pregnancy and lactation. Previous studies have also reported that female bonobos continued showing maximal swelling and copulate after conception for up to 6.5 months in the longest recorded case (Furuichi and Hashimoto 2002) or 3 months with a range of 1–6 months in six females (Douglas et al. 2016). Female bonobos resume sexual cycling during early lactation approximately 1 year (Kano 1992; Furuichi and Hashimoto 2002). 7.0 ± 4.8 months with a range of 3–14 (n = 5 females) in the study by Douglas et al. (2016). In this study, female bonobos exhibit maximal swelling within an average of 2.5 months after conception, and they resumed maximal swelling 240.5 ± 140.7 days (range, 32–516; n = 15) after parturition. In contrast, female chimpanzees resume sexual swelling when an infant is approximately 3–5 years old (Thompson 2005; Thompson 2013), and only one-third of mothers resume their cycles by the end of the second year (Thompson et al. 2012). Female bonobos resume sexual swelling much earlier than female chimpanzees do.

The inter-birth interval does not differ between bonobos and chimpanzees as much as the timing of resumption of maximal swelling does, although the inter-birth interval in chimpanzees is often longer than that of bonobos is (Table 2-2). The variation in inter-birth interval in chimpanzees is large and may change according to the population structure and habitat type. The slight difference in the inter-birth intervals between bonobos and chimpanzees is not entirely strange because although they belong to the same genus, their physiological characteristics can be dissimilar. Moreover, the weaning period does not differ

between them as much as the timing of resumption of maximal swelling does at approximately 3 years in bonobos(Kano 1992; Hashimoto 1997) and 4–5 years in chimpanzees (Clark 1977; Smith et al. 2013).

In chimpanzees, lactation infertility may occur similar to its reported occurrence in humans (McNeilly et al. 1994). Chimpanzees do not show maximal swelling during lactation and get pregnant shortly after they resume maximal swelling (Thompson 2013). Lactation infertility also may occur in bonobos because their inter-birth interval is similar to that of chimpanzees (Table 2-2). However, female bonobos resume maximal swelling during lactation infertility. Therefore, we wondered how the dynamics of their sex steroid hormones secretion change during this period.

Change of E1C concentrations and MSP based on days after parturition

After parturition, the blood concentration of prolactin is high for the duration of lactation and prolactin is released in response to tactile stimulation of the nipple (McNeilly 1979). As lactation progresses, the decrease in nipple contact decreases the amount of prolactin produced, thereby increasing the possibility of ovulation.

In chimpanzees, nipple contact is highest during the first 6 months and decreases afterward, but weaning is not completed until the infant is approximately 4–5 years old (Clark 1977). Change in nipple contact based on days from parturition does not differ between bonobos and chimpanzees during the first 2 years and frequent nipple contacts occur until the first 6 months (Lathouwers and Elsacker 2006).

In this study, we discovered that E1C levels during the follicular phase, length of MSP, and the proportion of MSP increased with an increasing number of days after parturition. These observations indicate that ovarian estrogen secretion started to increase based on days from parturition.

Studies in humans have shown that follicular growth is not completely inhibited during lactation amenorrhea (McNeilly et al. 1994). The possibility of ovulation increases with the progression of lactation. However, even if ovulation occurs during the lactating period, the ovulation often coincides with defects in the luteal phase and multiple ovulatory cycles do not continue (Howie and McNeilly 1982; Gray et al. 1990; McNeilly et al. 1994). In the amenorrheic lactation periods, the total estrogen excretion is lower than that in normal cycles and may cause abnormal follicular development (Howie and McNeilly 1982).

In bonobos, follicular growth may also occur during lactation periods. During the first 6 months after parturition, the maximal swelling did not occur likely because of intense nipple contacts. As nipple contacts decrease after the first 6 months, follicular growth may commence and cause the MSP to resume approximately 240.5 days after parturition. In this study, we found evidence of ovulation in a female with a 20-month-old infant. Douglas et al. (2016) also confirmed the occurrence of ovulation when an infant was < 2 years old. However, taken together with the inter-birth interval data, this observation appears to indicate that ovulation within the 3 years after parturition may not lead to a successful pregnancy. Bonobos of the E1 group conceived successfully within < 3 years from their previous parturition in only one case (n = 24, gestation period =7.7 months). Therefore, the inter-birth interval data suggests it is difficult to achieve a successful pregnancy for approximately 3 years after parturition.

Estrogen levels hardly reach the requisite level for a successful pregnancy within 3 years after parturition. Although we had only two samples, the E1C levels in successful pregnancy cycles appeared to be higher than those for unsuccessful pregnancy cycles. The same tendency was reported in chimpanzees (Thompson 2005). In this study, a possible case of early loss was observed. The early loss of pregnancy in humans is not uncommon, and 14–25% of pregnancies are terminated as early losses (Wilcox et al. 1990; Jones and Lopez

2013). The early loss of pregnancies in chimpanzees has also been reported in the wild (Thompson 2005) and in captivity (Littleton 2005; Wildman et al. 2011). E1C concentrations of the early loss cycle were as low as those of the non-conception cycle were, and significantly lower than those of the successful cycles.

Thompson et al. (2012) reported that female chimpanzees resume sexual cycling only after a sustained period to regain energy not only for milk production but also for the mothers to recover physically. Female bonobos resume sexual cycling earlier than chimpanzees do; however, they may need to wait a little longer before becoming pregnant to recover physically considering the finding that the inter-birth interval between live births does not differ between chimpanzees and bonobos (Table 2-2) ((Furuichi and Hashimoto 2002). Therefore, an important characteristic of female bonobos could be their resumption of maximal swelling even during early lactation periods.

Timing of ovulation relative to MSP

The timing of ovulation relative to the MSP has been previously discussed (Heistermann et al. 1996; Reichert et al. 2002; Douglas et al. 2016). In chimpanzees, most ovulation occurs during the MSP (Deschner et al. 2003; Thompson 2005). An association between ovulation and maximal swelling has also been found in other Old World monkeys with sexual swelling (See also Chapter 1 and 4)(Street et al. 2016). In contrast, previous studies of bonobos have revealed that one-third of ovulations do not occur in the MSP (Reichert et al. 2002; Douglas et al. 2016). In this study, however, ovulation occurred during the MSP in most cycles (92.3%). The high coincidence of ovulation with MSP may be partly because most cycles for which we could determine the timing of ovulation were those that occurred more than 3 years after parturition. As mentioned above, sexual cycling more than 3 years after parturition stabilized and maximal swelling often occurred.

Bonobos and chimpanzees belong to the same genus and, therefore, should share most physiological features. Because their inter-birth intervals do not differ (Furuichi and Hashimoto 2002), the time from parturition to the next conception would be similar between the two species. Female chimpanzees need time to recover physically after the energetic burden of lactation is lightened (Thompson et al. 2012) and, therefore, female bonobos may also require a similar physical recovery period before their next conception. Moreover, because it may be difficult for a mother to rear two offspring that are close in age simultaneously, female bonobos need to maintain an adequate inter-birth interval similar to that of female chimpanzees. However, although lactation infertility occurs similarly in both species, female bonobos resume sexual swelling much earlier than female chimpanzees do.

Tumescence of the sex skin in chimpanzees is directly related to the actions of estrogen (Ozasa and Gould 1982). The sexual skin of bonobos may have evolved to be more estrogen-sensitive than that of chimpanzees is and, therefore, maximal swelling may occur even at low estrogen levels. Maximal swelling outside reproductive purposes may have evolved as part of the unique socio-sexual features of bonobos compared with that of chimpanzees, including reduction of male sexual competition, high female social status, and the development of close female associations (Savage-Rumbaugh and Wilkerson 1978; Thompson-Handler et al. 1984; Kano 1992; Parish 1996; Furuichi 2011; Ryu et al. 2015).

2.6 Authors' contributions

Conceptualization, C. Hashimoto, H. Ryu; Investigation, H Ryu. Methodology, Mouri, K, Shimizu, K; Formal Analysis, T. Furuichi, H. Ryu; Writing – Original Draft, C. Hahimoto, H. Ryu; Writing – Review & Editing, C. Hashimoto, H. Ryu, T. Sakamaki, K. Mouri, K. Shimizu, T. Furuichi; Project Administration, C. Hashimoto, T. Sakamaki; Supervision, T.

Furuichi; Funding Acquisition, C. Hashimoto, T. Furuichi, H. Ryu (following Brand et al. 2015).

Table 2-1. Results of GLMM tests

Each table represents the results of the model and predictor variables. a) Effect of days from parturition on the inter-swelling interval (ISI). b) Effect of days from parturition on the length of the maximal swelling phase (MSP). c) Effect of lactation status (early lactation period; 240.5 days after parturition, late lactation; 240.5 days to birth) on the length of MSP. d) Effect of the swelling phase and years from parturition on E1C concentrations. e) Effect of the mother's age and offspring sex on the duration (days) from the last MSP to parturition during pregnancy. f) Effect of the mother's age and offspring sex on the duration from parturition to the earliest MSP. g) Effect of years from parturition on the proportion of MSP in observed days. h) Effect of days from parturition and successful pregnancy on E1C concentrations in the fertile phase (POP). i) Effect of days from parturition on the mean of the three highest E1C concentrations in all MSPs.

Models	predictors	est.	s.e	df	t	p
GLMM 1-a	intercept	35.12	5.89	8.46	5.96	2.7E-04
	days from parturition	2.2E-03	5.8E-03	6.76	0.37	0.72
GLMM 1-b	intercept	2.42	0.42	31	5.73	2.8E-06
	days from parturition	1.4E-03	4.7E-04	31	3.04	4.8E-03
GLMM 1-c	intercept	1.66	0.45	21.29	3.68	1.4E-03
	early lactation: late lactation	2.48	0.50	31.84	4.97	2.2E-05
	early lactation: pregnancy	1.17	0.81	32.79	1.45	0.16
GLMM 1-d	intercept	1.97	0.26		7.58	3.4E-14
	swelling 2: 1	-1.13	0.10		-11.14	2.0E-16
	swelling 2: 3	0.46	0.08		5.91	3.4E-09
	Infant age	0.01	0.07		0.16	0.87
GLMM 1-e	intercept	88.83	55.21	10.44	1.61	0.14
	offspring sex female: male	-21.16	21.79	6.55	-0.97	0.37
	mother age	1.5	1.99	10.11	0.76	0.47

Models	predictors	est.	s.e	df	t or z	p
GLMM 1-f	intercept	121.72	113.21	6.18	1.08	0.32
	offspring sex female: male	5.58	72.08	4.71	0.08	0.94
	mother age	4.58	3.88	3.60	1.18	0.31
GLMM 1-g	intercept	-2.24	0.11		-19.75	<2e-16
	years from parturition	0.38	0.01		26.01	<2e-16
GLMM 1-h	intercept	14.01	13.64	11	1.03	0.33
	days from parturition	3.6E-03	0.01	11	0.33	0.74
	pregnant (fail: success)	42.77	10.54	11	4.06	1.9E-03
GLMM 1-i	intercept	9.94	4.85	29	2.05	5.0E-02
	days from parturition	0.01	4.5E-03	29	2.36	2.5E-02
	pregnant (fail: success)	38.58	8.06	29	4.79	4.6E-05

Table 2-2. Inter-birth interval of bonobos at Wamba and that chimpanzees across study sites

Inter-birth intervals were calculated using data from surviving offspring. The data from chimpanzees in Kalinzu Forest for this study have been presented first, and the remaining data have been compiled by (Thompson 2013).

Community	Sub-species	Inter-birth interval (years)
Kibale (Kanyawara), Uganda	*P.t. schweinfurthii*	6.6 (2.4 – 8.2), N=21
Gombe, Tanzania	*P.t. schweinfurthii*	5.6 (3.3 – 8.1), N=74
Mahale, Tanzania	*P.t. schweinfurthii*	6.0 (3.3 – 8.1), N=116
Budongo, Uganda	*P.t. schweinfurthii*	5.2 (4.8 – 7.0), N=13
Kalinzu, Uganda	*P.t. schweinfurthii*	4.8 (3.0 – 8.9), N=27
Bossou, Guinea	*P.t. verus*	5.3 (1.9 – 10.7), N=21
Taï, Côte d'Ivoire	*P.t. verus*	5.8 (4.0 – 10.0), N=33
Wamba, DRCongo	*P. paniscus*	4.8 (2.4 – 8.2), N=24

Figure 2-1. Average E1C and PdG concentrations on days relative to the day of ovulation

E1C and PdG concentrations were indexed using the creatinine concentration (mg). Error bars show standard deviation. Day 0 is the ovulation day.

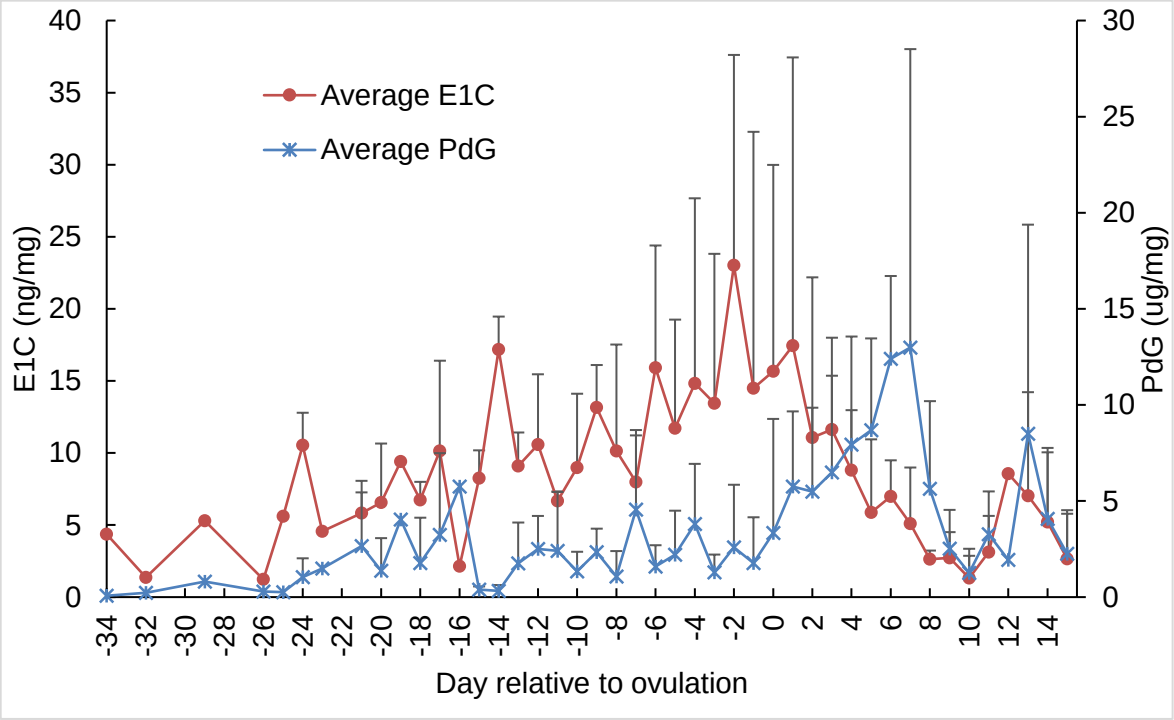
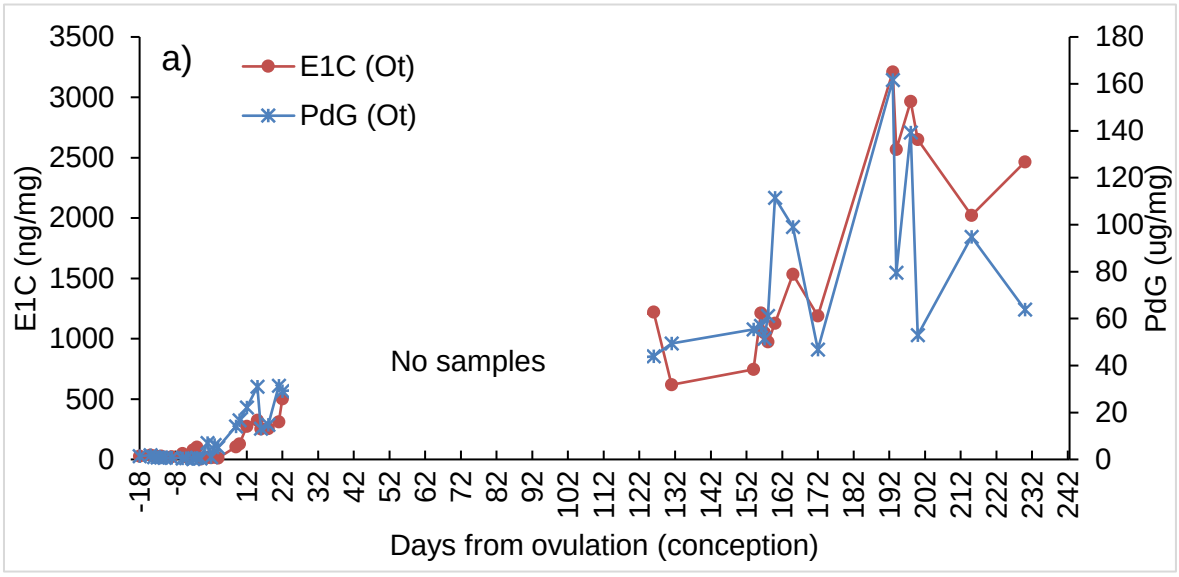


Figure 2-2. Changes in E1C and PdG concentrations during pregnancy

Two figures are showing urinary E1C and PdG concentration during successful pregnancies of two females (Ot and No). E1C (ng) and PdG (ug) concentrations were indexed using creatinine concentration (mg). Day 0 is the ovulation day which resulted in a successful parturition.

a) E1C and PdG profiles during pregnancy of Ot

b) E1C and PdG profiles during pregnancy of No



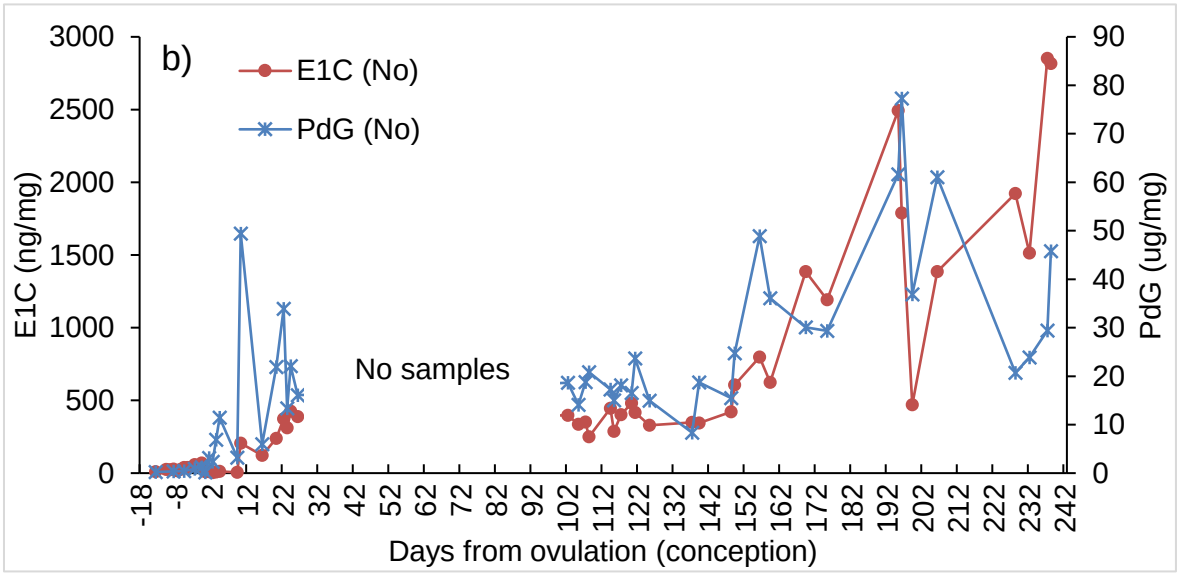


Figure 2-3. Changes in E1C and PdG concentrations from one cycle of presumed early loss

E1C and PdG concentrations were indexed using the creatinine concentration (mg).

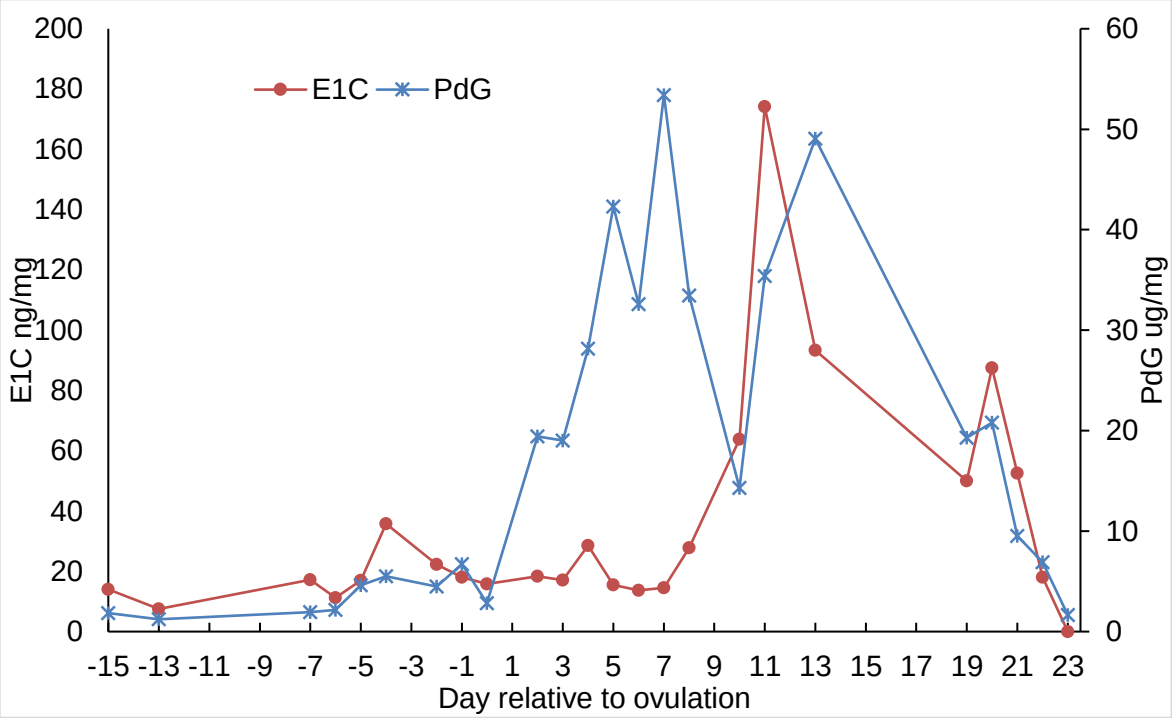


Figure 2-4. Duration of the maximal swelling phase (MSP).

Each data point represents the length of one MSP of a female at respective days from parturition.

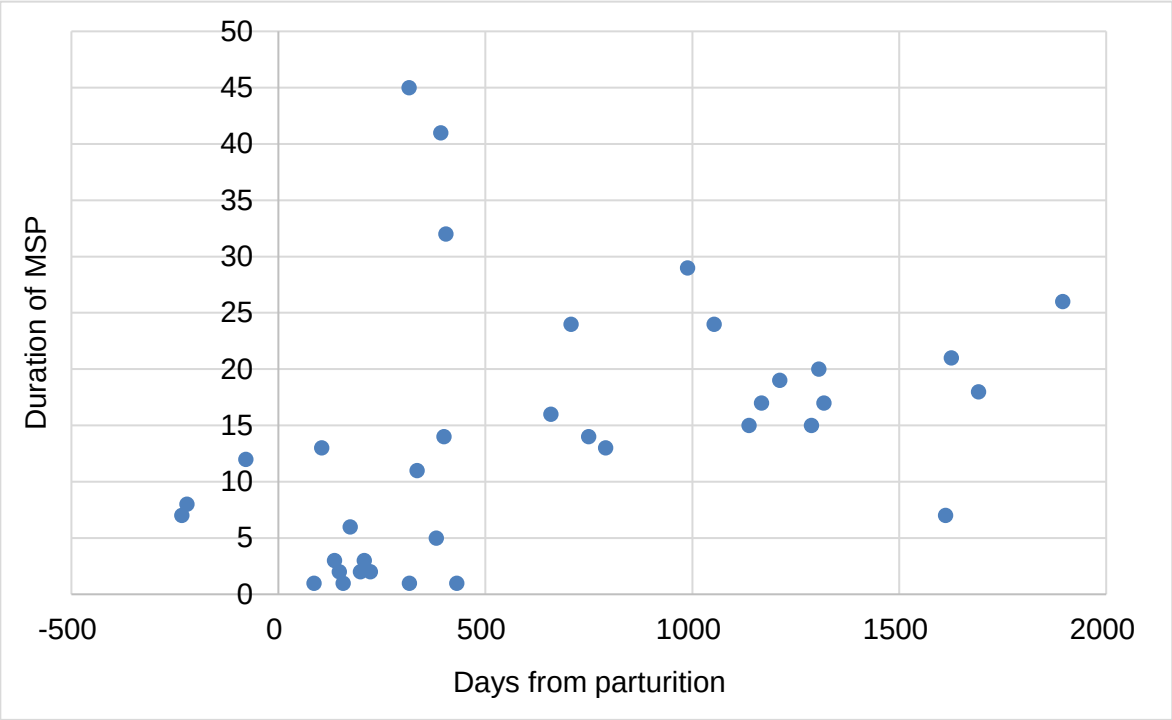


Figure 2-5. Mean E1C concentration of females in different swelling statuses in relation to years from parturition

The E1C concentration was indexed using the creatinine concentration (mg). Non-swelling is sw1, intermediate swelling is sw2, and maximal swelling is sw3. Data of sw1 from females with infant aged 2–2.5 years were not available.

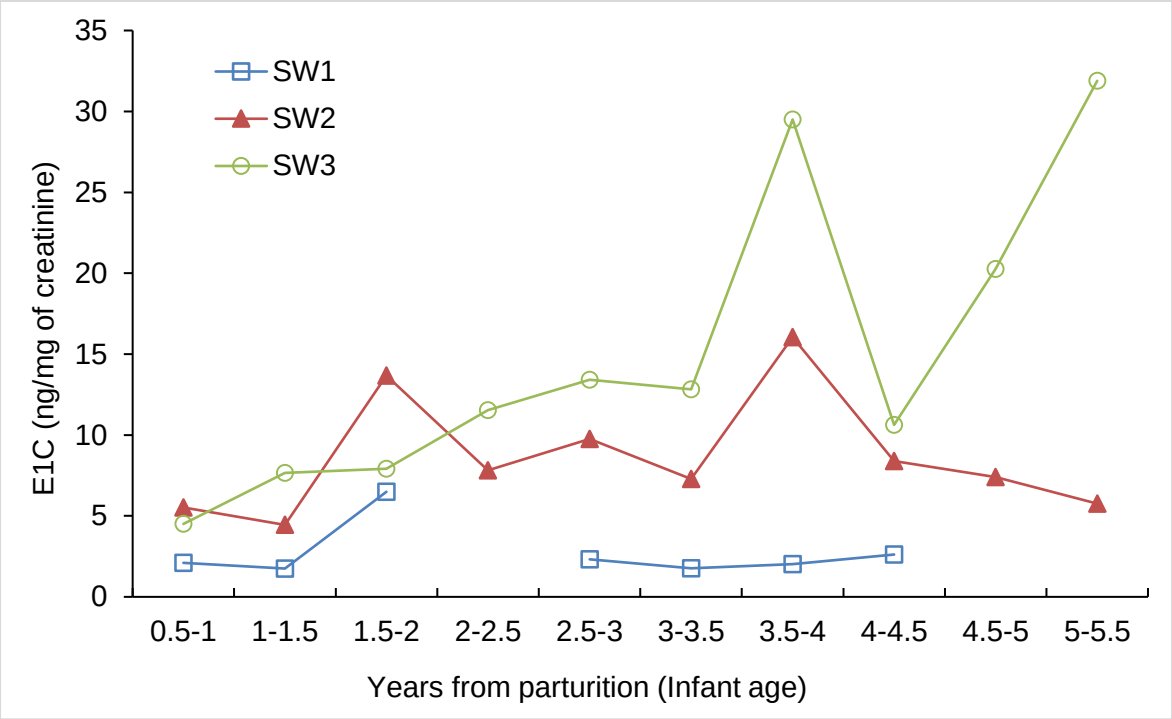
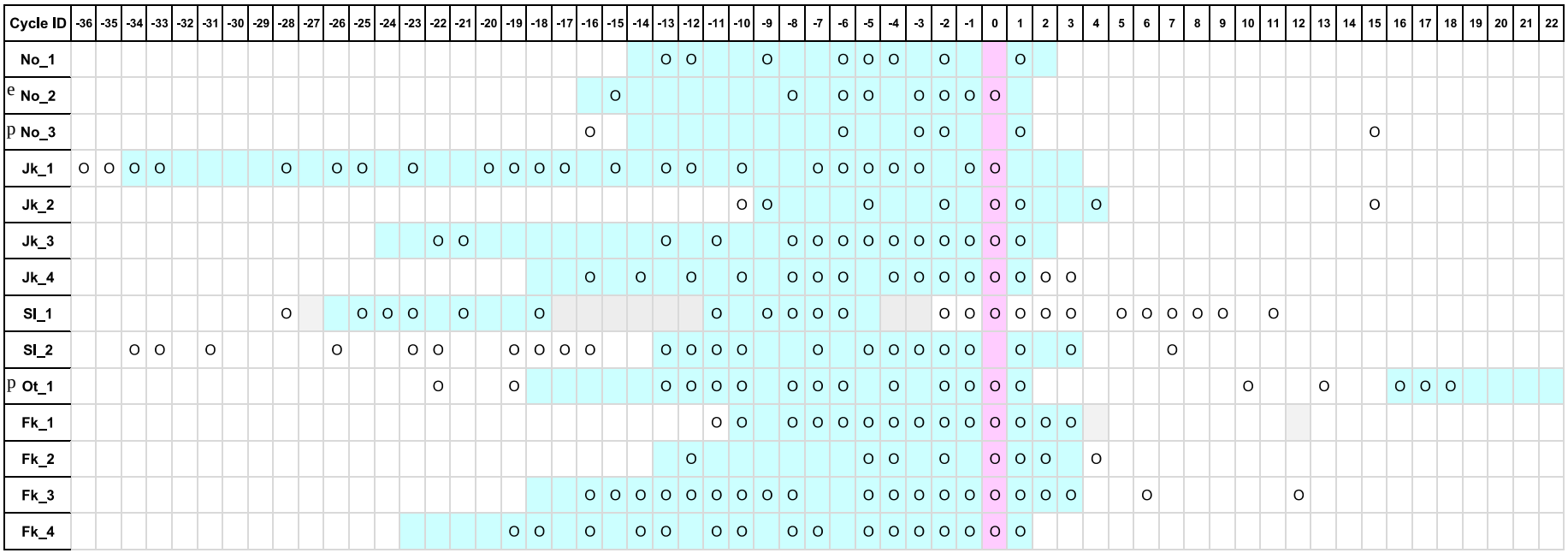


Figure 2-6. Timing of ovulation relative to the maximal swelling phase (MSP) in 14 cycles that was used to determine the day of ovulation

Blue cells show MSP, red cells show the day of ovulation, and circles show the days on which copulation was observed.



e: the ovulation cycle which resulted in early loss. p: the ovulation cycle which resulted in successful pregnancy (parturition)

Figure 2-7. Proportion of days on which each female was in the maximal swelling phase (MSP) at respective years from parturition

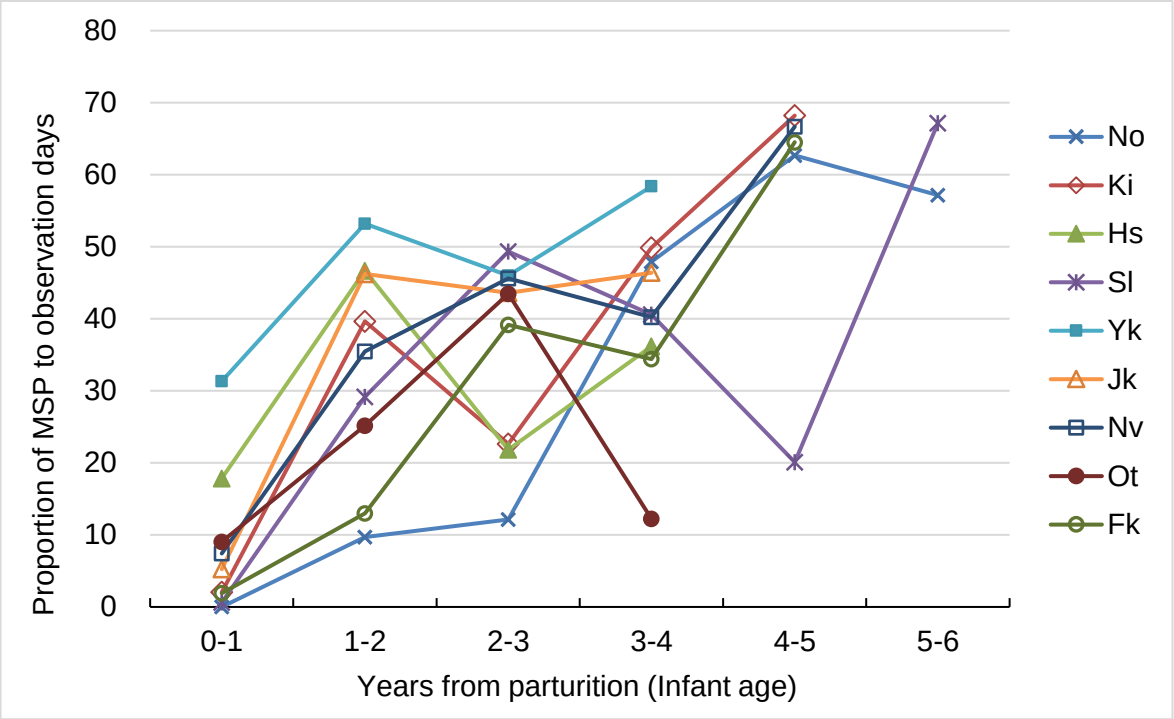


Figure 2-8. Changes in E1C concentrations during the fertile phase (3 days prior to ovulation and the ovulation day)

E1C concentrations were indexed using the creatinine concentration (mg). Each data point represents the mean E1C concentrations of the fertile phase at respective days from parturition. Triangles represent cycles leading to successful parturition, and the x mark represents a cycle that might result in early loss.

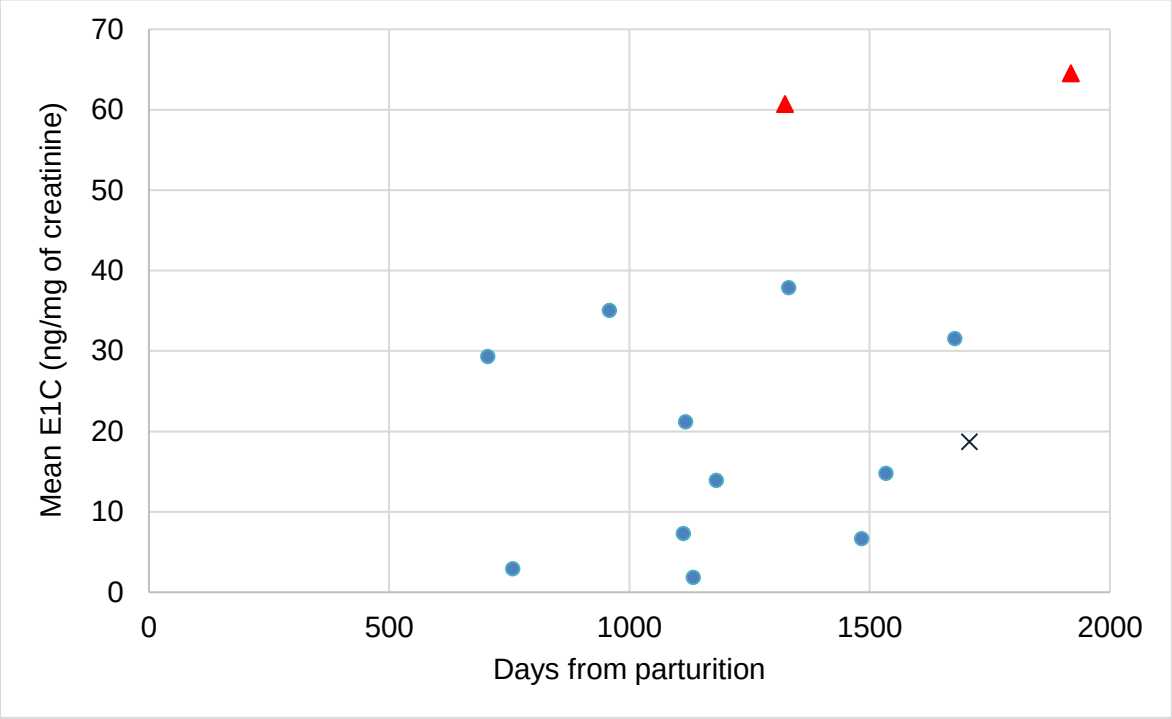
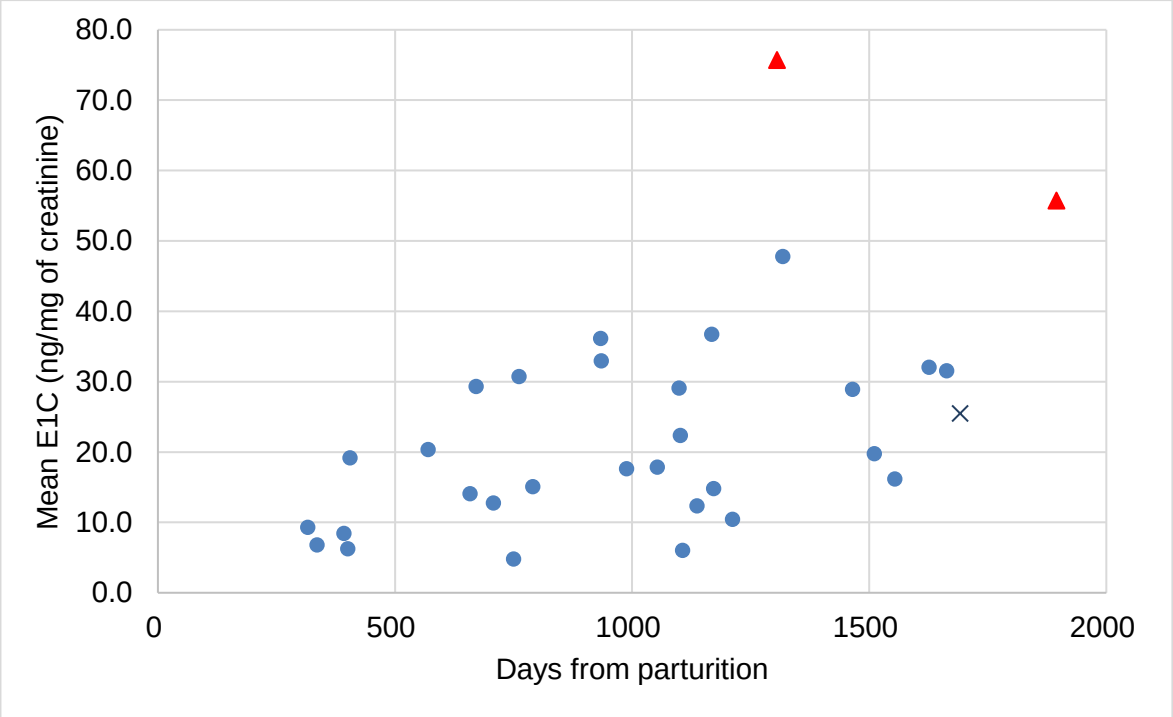


Figure 2-9. The mean E1C concentration using the three highest values of each maximal swelling phase (MSP)

Each data point represents the average of the highest three E1C concentrations of an MSP of a female at respective days from parturition. Triangles represent cycles leading to successful parturition, and the x mark represents the cycle which might result in early loss.



3. Chapter 3

Male bonobos determine the fertile phase of female bonobos depending upon sexual swelling and reproductive history

3.1 Abstract

One function proposed for the prolonged receptivity of female bonobos is that it lowers mating competition among male bonobos, allowing them to access a receptive female more easily than male chimpanzees. However, it is questionable whether being able to copulate with females more frequently actually reduces the effectiveness of mating competition. In this study, we tested the hypothesis that males can determine the fertile phase (3 preceding days of ovulation + days of ovulation) of females with some precision, since the ability to detect the fertile phase could greatly influence a male's reproductive success. Behavioral data and urinary samples of 9 females were collected from a unit-group of wild bonobos (E1) at Wamba, DR Congo from 2013 to 2015 for 14 months. The results from the behavioral observation and urinary sexual hormones (E1C and PdG) suggest that males selectively followed females who had a higher probability of ovulation. In particular, male rank predicted an increase in the number of copulations with the female with the highest probability of ovulation. High-ranking males also engaged in agonistic interactions more often when they intensively followed a given female. When there were several females with maximal swelling, group males were more likely to follow females with older dependent offspring, and whose maximal swelling phase was close to detumescence. Based on this finding, I proposed a modified way for ovulation probability calculation and a three-layered model for males' mate decision making in bonobos and other species for which ovulation is not completely concealed. Overall, this study suggest that males are able to discriminate the fertile phase of females, and male–male competition over access to females is also an

important mating strategy of male bonobos. However, it is still possible that frequent copulation of low ranking males with females with low reproductive quality plays some regulative role in male-male competition. Future studies, therefore, should examine more precisely how male bonobos match their sexual behavior with the probability of ovulation, as well as the role of females' high social status, in the sexuality of bonobos.

3.2 Introduction

Male competition for mates has a great impact on male behavior, physiology, and social systems (Andersson 1994). Bonobos (*Pan paniscus*) are well known for their peaceful and mild social characteristics (de Waal 1989; Furuichi 2011; Clay et al. 2016) compared with those of chimpanzees (*Pan troglodytes*). One hypothesis explaining their peaceful nature is their high operational sex ratio [(estrous sex ratio hypothesis; proposed by Furuichi and Hashimoto (2002)]. Based on findings that female bonobos are receptive for a longer period of time than female chimpanzees, this hypothesis proposes that the prolonged receptivity of female bonobo – achieved by prolonged and frequent maximal swelling phases – results in milder mating competition between male bonobos compared to chimpanzees. The main role of prolonged receptivity is therefore, in this hypothesis, confusing paternity as well as reducing the tension surrounding male reproductive success, allowing males to increase their chance of copulation. Prolonged receptivity is mainly a reflection of the maximal swelling during lactation periods – female bonobos achieve maximal swelling during the early lactation periods when the infant is younger than 2 years (Kano 1992), whereas female chimpanzees do not (Thompson et al. 2012), and even during pregnancy (Furuichi 1987; de Waal 1995).

Some researchers suggested that prolonged periods of maximal swelling might help females to conceal ovulation (Furuichi 1987; Reichert et al. 2002). In line with this

suggestion, female bonobos are known to have the longest period of maximal sexual swelling among species in that exhibit sexual swelling (Furuichi 1987; Kano 1992; Furuichi and Hashimoto 2002; Reichert et al. 2002). Moreover, the probability of ovulation during the maximal swelling phase in bonobos is the least predictable among these species (Reichert et al. 2002; Douglas et al. 2016; Street et al. 2016). The lower predictability of ovulation in bonobos might support the idea that the prolonged periods of maximal swelling functions to conceal ovulation. However, it is uncertain to what extent mating competition is reduced by easier access to mating due to the more number of receptive females.

The estrous sex ratio hypothesis assumes that males do not seriously care about producing offspring if they are able to copulate with any receptive females, or males are deceived by the signal of ovulation from the sexual swelling. However, considering that inter-birth intervals are not very different between bonobos and chimpanzees (Furuichi and Hashimoto 2002; Chapter 2), male mating competition, in terms of reproduction, should not be very different in the two species. Also, in previous studies on bonobos (Heistermann et al. 1996; Jurke et al. 2000; Reichert et al. 2002; Douglas et al. 2016), female reproductive history (lactation and parities) was not considered thoroughly as an important factor in determining the resumption and continuation of the menstrual cycle. It is possible that the maximal swelling phase less reliably indicates ovulation for females with younger infants (less than 2-years-old), as it varies in length a lot more than it does for females with older infants, or ovulation might not coincide with the maximal swelling phase for those females with younger infants (Chapter 2). Male bonobos may also respond differently to the sexual swelling of females with young infants, as those females might have a lower probability of getting pregnant, given that the inter-birth interval has a mean of 4.8 years (Chapter 2). If that is the case, then sexual swellings of females with young infants can be considered a less

meaningful source of information for male mating decision making, resulting in much variation in the predictability of ovulation within the maximal swelling phase.

Ovulation in bonobos, seems to occur at the second half of maximal swelling periods (Reichert et al. 2002; Fitzpatrick et al. 2015; Chapter 2; but see also Douglas et al. 2016). If sexual swelling reliably signals ovulation, male mating activity should have been selected to occur more frequently at the second half of maximal swelling periods in which the fertile phase (fertile window; Wilcox et al. 2000 or peri-ovulatory periods – POP; Goodall 1986; Deschner et al. 2003) is likely situated. In chimpanzees, male mating efforts, in particular, those of the alpha male who can exert most influence on male mating competition, were more concentrated on the fertile phase (POP) of females (Deschner et al. 2004; Thompson and Wrangham 2008). These findings are in line with the predictions from graded signal hypothesis, which implies that sexual swelling advertises a graded-signal of probability of ovulation so that it distributes males' mating attempts in relation to their social dominance (Nunn 1999; Fitzpatrick et al. 2015). Considering the genetic similarity (99.6%; Prüfer et al. 2012) and social structure (multi-males and multi females society with fission-fusion dynamics; Nishida 1968; Kano 1982; Aureli et al. 2008), it is likely that bonobo males can also determine the fertile phase of females with some precision. This is also expected if roles and mechanism of sexual swelling are conserved in bonobos, as found in Old World monkeys of which females have exaggeration in color and size of sexual swelling (Bercovitch 1988; Brauch et al. 2007; Daspre et al. 2009; Higham et al. 2012; Chapter 1).

In chimpanzees, males can adopt a coercive mating strategy or mate guarding to restrict promiscuous mating of females during their maximal swelling periods (Tutin 1979; Smuts and Smuts 1993; Muller et al. 2007; Muller et al. 2009). The efficiency of male mate guarding probably depends on the number of males in the group (Watts 1998) and the degree of synchrony of ovulation (Wallis 1985; Pereira 1991). Nonetheless, it is highly beneficial for

male chimpanzees if they can determine the fertile phase (POP) of females even within the maximal swelling periods, for better reproductive outcomes. However, coercive mating by males is missing in bonobos (Paoli 2009). Alongside which, the high social status of females (Parish 1994; Furuichi 2011) might facilitate female choice and prevent males from using a coercive mating strategy in bonobos as well. Therefore, male bonobos might deploy different strategies to increase their reproductive success compared with male chimpanzees. This different situation for male bonobos might result in a difference in male responses to females with differing probability of ovulation (probability of conception). However even though males' behavioral responses to fertile females is different in bonobos, it would be adaptive for male bonobos if they could judge the probability of ovulation and adjust their behavior in relation to a female's fertility. Following previous studies and the above assumptions, I hypothesize that male bonobos can determine the fertile phase of females with some precision ("males know" hypothesis). The fertile phase of this study is defined as 4 days of fertile window which includes 3 preceding days to ovulation and the day of ovulation (Deschner et al. 2003). This hypothesis predicts that;

1. Males' mating efforts, including intensive following (defined in methods section), copulations, intervention and solicitation of copulations will coincide with higher reproductive quality (based on the probability of ovulation and reproductive history) of females.
    a) Males will intensively follow the most fertile (close to ovulation and whose infant's age is older) female in the party when there are several females with maximal swelling.
    b) The number of copulations, intervention and solicitation of copulation will increase with the probability of ovulation and infant age.

2. Male agonistic interactions will increase when females get close to ovulation and females have older infants. This might also result in higher copulation rate between high ranking males and females with higher reproductive quality than for low ranking males.

To test this hypothesis and predictions, we first examined the hormonal profiles of females in maximal swelling. Second, we compared the male mating efforts and interests in relation to probability of ovulation and infant age (reproductive quality). Finally, we evaluated the relevance of the hypothesis and suggested a simple male decision making rule for mating effort allocation. This hypothesis will be supported if male bonobos can determine the fertile phase of female bonobos. If sexual swelling in bonobos is a reliable signal indicating ovulation, male mating efforts will be predicted from the onset or detumescence of maximal swelling phase. In particular, detumescence might be key to understanding male decision making, since an increase of serum progesterone concentration after ovulation results in detumescence of sexual swelling (Gillman 1940; Kato et al. 1980) and this seems to be also the case for bonobos (Reichert et al. 2002; Chapter 2). We will examine how detumescence of sexual swelling can be used to understand male mating strategy using a new way of calculating ovulation probability from both the detumescence and male intensive following behavior. Finally, we propose and discuss a decision making model for how males allocate their mating efforts toward females with different reproductive values for the best reproductive outcomes.

3.3 Materials and Methods

Study site and subjects

I conducted the study on a fully habituated free-ranging wild bonobo group, called E1 (for more details see Furuichi et al. 2012), 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 (Kano 1992). Researchers provisioned the group from 1976 to 1996. However, since research resumed in 2003 after the second Congo war, we have not provided any artificial food to the group. The group's home range consists of primary, secondary (including agricultural fields), and swamp forests (Hashimoto et al. 1998; Mulavwa et al. 2010). Although there is no clear division of dry and wet season, the amount of rainfall is lower in January and February than the other months and fruits availability is lower in these months (Mulavwa et al. 2008).

The study group (E1) comprised of 32 to 38 individuals, including 7 adult and 4 adolescent males and 9 adult and 3 adolescent immigrant females during the study periods (from 2013 to 2015). The age classes of females and males were defined following a previous study (Hashimoto 1997). Focal data were collected on 9 adult females (Table 3-1) and 7 adult and 3 adolescent males (Table 3-2). Three adolescent females who had no previous birth records were not the targets of focal sampling. Although one adolescent male became 8-years-old in October 2014, he was not the target of focal sampling (Altmann 1974), since he had not shown any sign of dominance challenges, which is a good indicator for adolescence (in chimpanzees; Pusey 1990). All of the adult females had immigrated into E1 group and had at least one successful birth after their immigration (for more details on life-history parameters of females see Sakamaki et al. 2015).

Behavioral observation

I collected behavioral data on adult males and females during three study periods (Study Period 1; September 2013 to February 2014, Study Period 2; July 2014 to September 2014,

Study Period 3; November 2014 to April 2015). Bonobos were followed from their bed site, located at around 6 AM, until they made a new night bed, usually after 5 PM. When they split into several parties, the largest party was followed as far as possible. To control for the effect of number of available group members, party composition was recorded every single hour following the one-hour party method (Hashimoto et al. 2001). Daily ranging party size and membership were defined as the number and identities of all individuals who were found during a given day when the total observation time of the day was longer than 2 hours. In total bonobos were followed on 255 days. However, I excluded two days on which I could follow bonobos for less than 1 hour. There was one day with very heavy rain so that no males were confirmed, and two days on which we could not see any females. These 5 days were removed from the data analysis, except to calculate the male dominance relationship.

For continuous focal sampling, one of the bonobos in the party was randomly selected based on a random order which was previously generated before field work began. I and one research assistant searched for the first target focal animal in the random order for at least 30 minutes, after bonobos were located in the morning. If we could not find the first one, we chose the second one in the order, and if we could not find the second one either, we moved to the next one and so on. To compensate for lack of focal sampling data due to the fission-fusion social system of the species (Kuroda 1979; Kano 1982), we also gave priority to individuals with less data for focal animal selection (see also Chapter 4). One focal session continued for 20 minutes and was terminated when a focal animal was out of sight for more than 5 minutes within a focal session. I only included the focal data in analysis when a focal session was conducted for longer than 15 minutes. After an individual was followed once, I did not conduct another focal sampling for the same individual before at least 100 minutes. I recorded all activities involving the focal animal, including feeding, moving, resting, and social interactions. To estimate neighboring individual patterns, the names and behaviors of

neighboring individuals within 5m of the focal animal were also recorded at 5 minute intervals, including a scan at the beginning of the focal session (5 scan points within a focal session).

All rare events, such as sexual and agonistic interactions, were also recorded as far as possible during party following. I recorded the individual ID of the participants of the interactions as well as time and duration. To minimize the sampling error caused by ad-libitum sampling, all copulations, genito-genital rubbings and agonistic interactions (e.g., bite, hit, displacing and chase), were recorded whenever observed by me and at least one assistant.

Ejaculation during copulation was not always possible to confirm. Therefore, a complete heterosexual intercourse without any interruption by others, was counted as copulation. The intervention of the copulation included direct aggressions toward the male who solicited a female for copulation or direct intervention of the copulation moment (interventions between males). Solicitation was counted only when the clear target was available. Male bonobos often have an erect penis during feeding while watching females, and so only the presence of erection was not counted as solicitation. For solicitation, an erection should coincide with other behaviors such as directed hand or body gestures (Graham et al. 2016) or shaking, bending, or removing branches or twigs to get attention from the target female.

I recorded agonistic interactions whenever there was a clear target, regardless of whether physical contact occurred. Therefore, undirected display behaviors, such as charging and branch dragging, were not considered agonistic interactions because there is no clear target of the behaviors (Vervaecke et al. 2000). When a counter attack was performed by the target right after (within 5 seconds) a directed aggression to the target, I considered it as part of the same interaction.

To evaluate males' interests toward females in the party, I defined male intensive following behavior as when a male keeps close distance (within 10m in 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 fulfilled these criteria, I considered this male was intensively following the target female and counted the number of males who did this on a given day.

In total, I followed bonobos for 1462.3 hours on 255 days (5.73 ± 1.25 hours per day). The total focal following time for all individuals was 280.22 hours (858 focal sessions; 14.75 ± 2.01 hours per individual) during the study periods. The average length of a focal session was 19.6 ±1.0 minutes and each focal session consisted of 4.88 ± 0.34 scans (4189 scans in 858 focal sessions).

Assessment of sexual swelling

Daily variation in sexual swelling of female bonobos was scored in relation to firmness and size of each individual's swelling (Furuichi 1987; Ryu et al. 2015). The sexual swelling status of females was assigned to one of three categories following a previous study (Furuichi 1987). Figure 1-1 (in Chapter 1) shows an example of changes in sexual swelling of one individual, named Fk. Swelling 1 (Figure 1-1a in Chapter 1) shows non-swelling status; swelling 2 (Figure 1-1b in Chapter 1) is for intermediate swelling status; and swelling 3 (Figure 1-1c in Chapter 1) represents maximal swelling status. 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 range of its minimum size. The swelling size variation of an individual between swelling phase 2 and 3 was sometimes not definitive. Therefore, firmness and shininess of the surface and movement during locomotion of the sexual swelling were the key features for

distinguishing swelling 2 and 3. The detumescent day was set to the first day when the maximal swelling started shrinking and lost its firmness and shininess. The maximal swelling phase was defined from the onset of the maximal swelling to the end of maximal swelling. When there is a temporal drop from swelling 3 to 2 which did not reached swelling 1 (the longest drop from 3 to 2 was 6 days), we considered the maximal swelling phase continued (Chapter 2).

Since this study followed a conventional method (Furuichi 1987) for scoring sexual swelling stages, there could be subjective bias on scoring between observers. To reduce observation errors and bias on sexual swelling scoring, at least one researcher and two research assistants discussed the sexual swelling status of each female during field observations at the daily meeting every evening (Ryu et al. 2015). Then we determined the swelling score of each female for the day.

Urine sample collection

Urine was collected using filter paper (Whatman #1 Ø 5.5cm) while the largest party was being followed. When a female bonobo urinated in the canopy, the urine usually dropped on leaves of terrestrial plant or small trees. After a female finished urination, I or one of two trackers collected urine. We put the tip (around 1/4 of the whole filter paper) of the filter paper into the urine drops and waited until it absorbed to around 2/3 of the paper. By putting only the tip of the paper into urine, we avoided contamination of dirt from leaves. It is usually not possible to collect the urine of female bonobos when they urinate on the ground. However, when the urine is available on fallen leaves on the ground without any contamination, we collected the urine. Not to disturb the bonobos, we collected urine after the bonobos left the place so that we could keep at least 5m distance between the collector and bonobos. When the urine was mixed with an individual's own feces or another's urine or

feces, we did not collect the urine. After I came back to the camp, I put the urine sample papers into a dry-box which contained 500g of silica gel until the paper dried. The paper usually fully dried within 2 days, but to make sure, I kept the paper in the dry-box for at least 5 days. I also changed the silica gel every week and heated the used silica gel in a hot pan for more than 30 minutes to reuse it. This allowed us not to waste any silica gel. After each paper was removed from the dry-box, they were individually separated using plastic zipper bags and those individually packed zipper bags were put into a big zipper bag with silica gel and stored in a dark room. The longest period that the urine was stored at room temperature was 6 months. After the samples were moved back to Japan, I stored them in a freezer (-20℃) until the moment of urine extraction (Knott 2005). A previous report showed that, using this method, the estrogen and progesterone metabolite are not changed until after 6 months at room temperature (Knott 2005).

Hormonal measurement

In total, I ran enzyme immunoassay (EIA) on 1090 urine samples (425 samples by trackers, 665 by me). When there were 2 or more urine samples in a day for the same individual, I used the urine sample that was collected earlier whenever creatinine concentration was higher than 0.8mg/dl. After EIA, I discarded 201 samples and used 889 samples (344 samples by trackers, 545 by me) for further data analysis. Among removed samples, 138 samples showed low creatinine concentration (lower than 0.8mg/dl). Thirteen samples were duplicates in the same day and 49 samples had too little (absent) or too high PdG and E1C concentrations (25 samples by trackers, 24 by me) which may have been caused by infant urine or identification confusion. These possible errors in identification can be a serious problem, so I checked the 49 samples and found that among them there were only 4 samples with very high concentration of E1C and PdG which were caused by

identification confusion of the individual with a pregnant individual. The other 46 samples had no PdG and E1C probably caused by urine from their infant since all 9 study subjects had dependent offspring during the study periods. In the cases when coefficient of variation (CV) is higher than 20%, I retested all the samples. When there were too many days between samples for one individual, I excluded the urine from data analysis, since the urine sample is merely meaningful to determine menstrual cycles.

After I chose 889 samples, I discarded 194 urine samples from the pregnant individuals and 36 samples from one female (Sl; sampling gap was too large to determine ovulation) for ovulation detection. Therefore, in total only 660 samples were used to determine ovulatory cycles. More details on the number of urine samples and examined swelling cycles are available in Table 3-1.

Urine was extracted from the filter paper using deionized water with mechanical shaking, following an unpublished methods developed Mouri and Shimizu (Mouri and Shimizu 2014; more details in the Chapter 2). I measured creatinine concentration of the samples using Jaffe reaction methods (Taussky and Kurzmann 1954). I did not dilute the extracted urine samples in the first assay. However, when creatinine concentration of the samples was higher than 3mg/dl, I retested them by diluting the samples. Urinary E1C and PdG concentrations were measured by enzyme immunoassay (EIA) which is formally described in the previous study using captive chimpanzees with modification (Shimizu et al. 2003a). I used antibody (Cosmo Bio in Japan) against estrone-3-gluclonide BSA, pregnanediol-3-glucuronide BSA, and horse radish peroxide conjugated steroid derivatives (Cosmo Bio in Japan) for EIA. For more details regarding the EIA, please see Chapter 2. The sensitivity of EIA was 6.6pg/ml for E1C and 2.1ng/ml for PdG and if the concentration was below these values we excluded that sample from analysis. Inter plate CV for E1C was 10.79 and 15.83 for PdG. Intra sample CV was 5.66 for E1C and 6.66 for PdG. ANCOVA tests with the different dilutions from three

different bonobo urine samples (N = 3x4) confirmed that there was no serious violation of parallelism between the diluted samples and the standards (*P* values ranges 0.155 to 0.337 for E1C and 0.255 to 0.730 for PdG). The recovery test by adding fixed amount of standard solutions to three different samples (N = 3x4) showed high regression coefficient values (E1C: $Y = 0.99X + 0.01$, $r^2 = 0.985$, PdG: $Y = 0.99X - 2.5$, $r^2 = 0.967$), which suggests that E1C and PdG were successfully recovered in assays.

Defining the fertile phase (peri-ovulatory periods) and probability of ovulation and conception on a given day within a cycle

The date of ovulation was estimated based on sustained urinary PdG rise above the baseline (see Chapter 2). The baseline was defined as the mean of urinary PdG concentration of the 10 days preceding a given day. The sustained PdG rise (threshold value for ovulation detection) was determined when the PdG concentration on the given day was more than two standard deviations above the baseline for 3 successive days within a week. For more details, please see Chapter 2.

I calculated the 4 day fertile window, which starts from 3 days prior to ovulation, based on findings that sperm are most fertile within three days in the reproductive tracts and eggs are most fertile within 24 hours after ovulation (Wilcox et al. 1995; Dunson et al. 1999). This criterion to determine the fertile phase has already been used in several studies in several primate species including wild chimpanzees in Taï (Deschner et al. 2003) and wild bonobos in Luikotale (Douglas et al. 2016).

To calculate the probability of ovulation on a specific day within a cycle, I used the following equation proposed by Deschner and his colleagues (Deschner et al. 2003). When the onset of maximal swelling phase was not observed (6 cases) or the detumescent day was not observed (4 cases), the start day and/or the end day was determined as when a female was

first observed with maximal swelling and/or the last day when she was observed with maximal swelling (see Figure 2-6).

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

In this equation, *t* refers to a specific day within the maximal swelling phase which starts from the onset of maximal swelling; n_t is the total number of cycles in which ovulation occurred on day *t*; and *n* is the total number of ovulatory cycles of this study.

I also calculated daily probability of conception (the probability that the sperm can meet the egg) using the equation from the same study (Deschner et al. 2003).

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

$P(X(f) = 1)$ refers to a probability of conception on a given day (*f*) within the cycle. Since I already determined the probability of ovulation – $P(T = t)$ using the previous equation – the probability of conception is the sum of the probability of ovulation on the given day (*f*) and the following 3 days of the given day (*f*). By following the same equation used in the same species as had been done previously, we can directly compare our results between species, as well as between populations of the same species.

Estimating male dyadic dominance hierarchy

I generated male dominance metrics based on dyadic agonistic interactions. Agonistic interactions with physical contact included biting, hitting, and trampling. Agonistic interactions without any direct physical contact included chasing, charging, and directed display (sometime with branch dragging). I only used dyadic agonistic interactions which had a clear outcome of loser and winner. Loser is defined when the recipient of the aggression showed submissive behaviors such as grimace, screaming, running away, or retreating. I did not include interactions when the recipient did not show any response or when there was a

counter-aggression from the recipient right after (within 5 seconds) the initial aggression. In total, I observed 844 dyadic agonistic interactions. Only 16 dyadic interactions were followed by counter-aggressions. Therefore, in total 828 dyadic interactions (p1: 314, p2: 162, p3: 352) were selected to calculate male dominance hierarchy.

I used the 'steepness' package (Leiva and De Vries 2014) to build dominance hierarchy and David's score. I also checked h′ index (de Vries 1995) – modified version of Landau's linearity index (h index in Landau 1951) – using the 'igraph' package (Csardi and Nepusz 2006) following Shizuka (2016) to decide whether the male dominance hierarchy is linear or not.

Behavioral data analysis

I used R 3.2.4 Revised (R Core Team 2016b) with R studio 0.99.893 (RStudio Team 2015) for all analyses. I used generalized linear mixed models with negative binomial error distributions along with several packages including lme4, lmtest, and lmertest to fit our models to the data. More details of each model will be described later in this section. I also checked multicollinearity between independent variables in the model based on variance inflation factors (VIFs) after we ran all mixed models. In all models, the maximum VIF were less than 2, therefore the multi-collinearity among the predictor variables are of serious concern (Zuur et al. 2010). The response variables are the daily counts of copulation, aggression, and solicitation and intervention of copulation, as well as the number of males on a given day who intensively followed a certain female. To control the repeated measure of the behavior, female ID or the ID of the female-male dyad was integrated into the model as a random variable. Date of observation was not included as a random effect because two random effects showed convergence warning in some models.

In bonobos, it was reported that a male's copulation rate is related with other social factors, such as mother's supports and relative dominance rank caused by changes in party composition in a unit-group, or community (Surbeck et al. 2011). Therefore, we may need to consider those factors as predictor variables in the models (Surbeck et al. 2011). However, the 4 males who had their mother in the group, were almost always with their mother in the party. There were only 23 out of 744 days (on which males were observed) that any of the 4 males were found without their mother (only 3.09%; sum of days of the 4 males). Therefore, I did not include this variable in the models. Fluctuation of the relative rank between days because of change in party composition was not the main predictor, since our question is more generally whether the male rank within the community is an important factor for understanding male mating efforts. Also the alpha male was the highest ranking male on 188 days of the 253 observation days on which we observed males (74.3%). The beta male occupied the highest position on 33 days (13.0%), the 3rd ranking male for 9 days (3.6%) and the 4th for 17 days (6.7%).

I also considered that the reproductive quality of female bonobos has two main components. One is the increasing chance of getting pregnant with the growth of infants over time, regardless of the menstrual cycle on a wide time scale. The other is changes in the probability of fertility (the chance that fertilization – a sperm meets an egg) within a menstrual cycle based on changes in the probability of ovulation. It is likely that these two components are closely related with each other given that the energetic constraint from lactation has a regulative role in the menstrual cycle, and thereby also reproduction in female chimpanzees (Thompson et al. 2012). This is probably similar to female bonobos, although bonobo female resume their swelling cycle earlier (Furuichi and Hashimoto 2002). Therefore, it is reasonable to use infant age, as a proxy for female body condition, to estimate the female reproductive quality.

The chance of fertilization increases until to the day of ovulation. Therefore, I considered the number of days from ovulation in the menstrual cycle as a predictor variable. The day of ovulation was coded 0 and one day before the ovulation was -1 and, two days before was -2 and so on. The days after the ovulation were 1, 2 and so on. In some analyses, I used the data from -14 days before an ovulation to the ovulation day to clarify the effect of the probability of ovulation on male mating efforts.

Infant age was coded in ordinal scale by putting together 6 months as 0.5 years. It ranges from -1 to 5.5 in the data set. From the day of birth to 6 months, the infant age was coded 0.5. From six months before parturition to the day of parturition were therefor coded -0.5. I coded -1 from the detumescent day of maximal swelling which coincided with ovulation which resulted in successful pregnancy. Finally, to avoid the convergence errors of the model, I rescaled all of the predictor variables (male rank, days from ovulation / detumescence /onset, infant age, the number of males and females) from 0 to 1 from the original scale. However, if I used the scaled predictors in the figures, it might not be intuitive. Therefore, the figures were made with the original scale of the variables.

Male intensive following after a female with different reproductive quality

To examine whether or not the number of males per day who intensively followed a certain female was related with ovulation probability and infant age, I ran a series of generalized linear mixed models following the hurdle model approaches (Zuur et al. 2009). In the first model, I tested whether or not maximal swelling and increase in infant age predict the number of males who intensively followed a certain female (GLMM 1-1) using 250 days of focal party following. One row of the data set comprised swelling status (ordinal 1 to 3) and age of infant of a certain female as well as her own age on a given day. In the same row, the number of males and females with maximal swelling of the given day were placed. These

two variables were also included as fixed effects to examine whether or not the spatio-temporal distribution of males and females on the day influences the number of males who intensively follow the female. There were on average 6.4 ± 2.4 females in a day.

In the second model, I took a subset of the data, which only consisted of the females with maximal swelling, to examine the effect of detumescence of maximal swelling and infant age (GLMM 1-2) more specifically. This subset consisted of the 205 days on which at least one female with maximal swelling were present. The average number of females with maximal swelling per day was 2.7 ± 1.3 (range: 1 to 8 females with maximal swelling per day).

The third model (GLMM 1-3a) of the hurdle models was similar to the second model, except that I only used the data from the maximal swelling phase for females whose ovulation date was determined (138 days; 14 cycles in 5 females). However, the effect of ovulation on the model could be an artifact of too many zero responses from males on days far from ovulation. To check this possibility, I again made a subset of the data, which set the ovulation day from -14 to 0. Then, I ran a model with this subset (GLMM 1-3b) and compared it with the results from GLMM 1-3a. Fourteen days from ovulation was selected because the average length of maximal swelling phase was 14.0 ± 11.2 days, although some maximal swelling phase continued for more than 20 days (Chapter 2). The number of observation days was reduced to 104 days in the GLMM 1-3b, and each day an average of 1.4 ± 0.5 females with maximal swelling were observed (range: 1 to 2).

Since data collection was neither completely random nor completely all occurrence, it is possible that the temporal relationship (autocorrelation or spatio-temporal dependency) between sampling days might result in poor estimation of the models. To check this possibility, I conducted a simulation approach with random sampling from the smallest data set, in which the days of males' intensive following was observed (95 days among 250 days) and only consisted of females with maximal swelling. As a result, there were on average 3.1

± 1.6 females with maximal swelling for those 95 days (45 maximal swelling cycles from 9 females).

With this smallest data set, first I ran a mixed effect model with binomial error distribution with the data from all 95 days (GLMM 1-4a). The response variable for this model was the number of males who intensively followed a certain female on a given day, relative to the number of males not involved in intensive following on the same day (linked by 'cbind' function). In this model the response variable, linked by 'cbind', used the odds between the two variables and examined whether or not the probability (more exactly the odds ratio) of male intensive following is predicted by predictor variables. This model comprised infant age, number of days from the onset of maximal swelling, number of females with maximal swelling, and number of males that day. Here, I used the onset of maximal swelling to test one of the predictions that males more likely choose to follow the female whose sexual swelling had started earlier when there are more than 2 females with maximal swelling. Also, because the detumescent days were already used in the previous three tests, it seems not very meaningful to test it again here with the same predictor. The reason I used the binomial error distribution in this model was to make the response variable of GLMM 1-4a identical to the next random sampling simulation model (GLMM 1-4b). It also reduced the running time of the simulation and reduced the convergence error from using negative binomial function of the lme4 packages.

To run the simulation model, first I randomly sampled 45 days from the 95 days (because 45 maximal swelling cycles were used) of the data which used for GLMM 1-4a. I repeated this random sampling model 100 times and averaged the coefficient values of the models without any convergence warning.

Other mating related behaviors of males including copulation, intervention and solicitation of copulations, and total aggression of males

Male behaviors including copulations and interventions of copulations, and solicitation of copulations are probably directly related with male mating efforts. To examine the number of these three events between male–female dyads on a given day, three separated generalized linear mixed models with negative binomial distribution were conducted. For these three analyses, I used the 14 determined ovulatory maximal swelling phase – days from -14 to 0 from the ovulation date (0 is ovulation day) – to examine the effect of probability of ovulation. This data is the same data set with the GLMM 1-3b, which comprised 104 days of observation. Within this maximal swelling phase (in 104 days of observation), there were temporal drops from maximal swelling to intermediate swelling (swelling score 2). The maximal swelling temporally dropped to the intermediate swelling on 11 days from 5 females (11 female-days). These 11 female-days were excluded from data analysis. However, within the menstrual cycle of a female called Sl, 3 days of the fertile phase were outside of the maximal swelling phase. These 3 days of intermediate swelling phase remained in the data set.

All three models were fitted using negative binomial error distribution (copulation: GLMM 2-1a, intervention: GLMM 2-2, solicitation: GLMM 2-3). Each model has 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 were controlled by the hours that the male and female of the given dyad were observed together (the counts of one-hour party on a given day) using the offset function. The predictor variables were the number of days from ovulation, the infant age of the female of the dyad, male rank, female age, and male age.

Young bonobo females engage in more copulations than old females (Ryu et al. 2015), so female age was included in the model to evaluate its influence on copulation, in particular for the copulation model (GLMM 2-1a). In bonobos, it has been shown that males who have their mother in the same party have better copulation opportunities (Surbeck et al. 2011). Also, the existence of their mother in the group might influence male rank (Furuichi 2011; Tokuyama and Furuichi 2016) and the probability having a mother in the group is probably higher for younger males. Therefore, to include these social dynamics to some extent into the model, male age was included in all three models.

It is possible that the reduced data set (only from -14 to 0 to the ovulation day) might not represent the general pattern of copulation of males in the study group. To confirm this possibility, an additional negative binomial error GLMM (GLMM 2-1b) was conducted. In this model, the response variable was the number of copulations of the day controlled by one-hour party presence time (in offset term). I included male rank and male age as the predictor variables.

I also examined the total number of aggressions for each male on a given day using all 250 observation days (GLMM 2-4) since male–male aggression might be related both directly and indirectly with the male mating competition. The total number of male–male aggressions on a given day was fitted using generalized linear mixed model with negative binomial error distribution. In this aggression model, the female ID, for the female who was intensively followed by males on the given day, was the random effect. However, there were 6 days (among 250 days) when two females were the targets of male intensive following. For these 6 days, I set the ID of the female who was followed by more males as the random effect. One row of the data sheet represents the total number of aggression of the day (the response variable). The predictor variables were the number of males, number of females

with maximal swelling, and existence of male intensive following (observed or not on a given day).

3.4 Results

From the daily sexual swelling record and using 657 urine samples, I was able to determine 14 ovulatory cycles and 5 anovulatory cycles in 19 maximal swelling cycles from 7 females (Table 3-1). The 14 ovulatory cycles were only found in 5 females whose infants were more than 2.0 years old. The earliest ovulation of females during study periods was for a female (Jk) whose infant was 24-months-old. There was one possible case of ovulation from a female (Nv) whose infant was 20-months-old. However, the exact date of ovulation could not be determined because of a 5 day sampling gap, and so this case was not included in data analysis.

Male dominance hierarchy

During Study Period 1, one male named JR temporally occupied the highest rank of the group (Table 3-2a). In this period, however, the h′ index was 0.84 which indicates an unclear linearity of the male dominance hierarchy (Martin and Bateson 1993; Surbeck et al. 2011). During Study Periods 2 and 3, JR received a lot of aggression from the top 3 ranking males and his rank dropped to 5th (Table 3-2b). To take into account this fluctuation in dominance hierarchy for analysis, I separated the male dominance hierarchy in Study Period 1 from Study Period 2 and 3 (Table 3-2).

Male intensive following behavior toward females

In total, male intensive following behavior after females was observed on 95 days among 250 observation days. The number of males who intensively followed females during a day

ranged from 0 to 8 (1.4 ± 2.1 males per day). Among these 95 days, there were 6 days when two females were the targets of the male intensive following. Using the data from all observation days (250 days), I confirmed that the number of males who intensively followed a female increased when the target female had an older infant and maximal swelling (Figure 3-1a, 3-1b; GLMM 1-1 in Table 3-3). Female age, which probably reflects the parity of females, did not explain the number of males who intensively followed females. The number of males of the daily ranging party and the number of females with maximal swelling (FMS) were not responsible for the increased number of males in intensive following after females (GLMM 1-1 in Table 3-3). Interaction between infant age and swelling score was not significant.

With the reduced data set, which comprised the data only from females with maximal swelling (GLMM 1-2 in Table 3-3), I found that the number of males who intensively followed females increased when the sexual swelling of females was getting close to detumescence (Figure 3-2a). Infant age was also positively correlated with the number of males engaged in intensive following (Figure 3-2b). The other predictor variables were not responsible for the number of males engaged in intensive following. Importantly, it should be noted here that this model revealed a significant interaction between the days from detumescence and infant age. This means that, the increase in the number of males in intensive following close to the day of detumescence was more rapid or less rapid (or the slope of increase was steeper) depending upon the age of the infant of the target female (Figure 3-2c).

The results did not change when I ran the model (GLMM 1-3a in Table 3-3) only with 14 ovulatory maximal swelling phase (5 females in 138 days). The number of males who intensively followed a given female, increased when females were near to ovulation (Figure 3-3, GLMM 1-3a), as well as with the increase of infant age of females (GLMM 1-3a). The

other predictor variables did not predict the number of males intensively following the given female. The interaction between the probability of ovulation and infant age was not significant. This may be related to the small size of the data set, or it could be that males can indeed detect ovulation precisely so that they follow a female regardless of her infant's age when the female has an ovulatory maximal swelling cycle. With the reduced data set (GLMM 1-3b in Table 3-3) – only including 14 days from ovulation to the day of ovulation to avoid the many zero responses of early maximal swelling phase – I was able to confirm the same results with GLMM 1-3a. In GLMM 1-3b, I confirmed that the number of males engaging in intensive following of a given female increased when the days were close to ovulation and with infant age. There was no significant interaction between these two variables. However, the number of females with maximal swelling also predicted the number of males who intensively followed (GLMM 1-3b). This might just be that, in the reduced data set, the spatio-temporal distribution of females with maximal swelling coincided with the increasing number of males engaged in intensive following, or it may reflect temporal synchrony of sexual swelling phase in bonobos, if it does exist.

Lastly, the model with only the days when male intensive following was observed (95 days, with 45 maximal swelling cycles from 9 females) confirmed that more males intensively followed a female when the female had an older infant and the days from the onset of a maximal swelling phase was further (GLMM 1-4a in Table 3-3, Figure 3-4). The number of males on a given day was negatively related to the number of males in intensive following of a given female. The number of females with maximal swelling also had a negative influence on the number of males who were in intensive following (GLMM 1-4a). The interaction between number of days from the onset of maximal swelling and infant age was also significant in this model, as found in GLMM 1-2. Using the 100 times of simulation data, I consistently found that more males intensively followed the female whose maximal

swelling started earlier and whose infant was older (GLMM 1-4b in Table 3-3). The interaction between the onset of sexual swelling and infant age was also significant. However, this model did not show the negative effects of the number of males and number of females with maximal swelling on the day (GLMM 1-4b). This suggests that the effects of the two predictor variables were caused by some bias in the reduced data set of GLMM 1-4a.

Male's copulation related behaviors in relation to ovulation

Ten males copulated with the 9 female subjects 570 times in 250 days (Table 3-1b). The frequency of copulation was 0.066 times/hour/male for males (570/8609 of total presence counted by one-hour party attendance; party presence count) and 0.070 times/hour/female (570/8137) for females. Among 570 copulations, 492 copulations were observed from females with maximal swelling (86.3%). Neither male rank (N = 10, Spearman's rho = -0.217, p = 0.576) nor male age (N = 10, Spearman's rho = -0.147, p = 0.560) was correlated with the number of copulations with females with maximal swelling. I observed 440 solicitations of copulation from a male to a female (regardless of success or failure) in 250 days (0.051 times/hour/male; 440 in 8609 party presence counts). Intervention of copulation was observed 80 times in 250 days (0.009 times/hour/male; 80 in 8609 party presence count). Male-male aggressions, including dyadic (also tied aggressions) and involving more than 2 individuals, were observed 873 times in 250 days (0.101 times/hour/male; 873 in 8609 party presence count).

Days from -14 to 0 from the ovulation date of the determined ovulatory cycles (14 cycles from 5 females), 209 instances of copulation within 48 dyads in 104 observation days (2 mother–son dyads were excluded) was observed. The frequency of copulation was 0.041 times/hour/dyad (in 5040 dyad-party hours; total presence count of a dyad found in the same party). I observed 201 instances of solicitation in 5040 dyad-party hours (0.040

times/hour/dyad) within the 48 dyads in the 104 days. Intervention of copulation between males was observed 42 times in 5040 dyad-party hours (0.008 times/hour/dyad). The number of male–male aggressions in the 104 days were divided by the party presences counts of males as done in the previous paragraph. In total, 472 male–male agonistic interactions were observed on 104 days (4203 party presence counts in total) and the frequency was 0.112 times/hour/male (472/4203).

The number of copulations increased when the ovulation day of a given female was getting close (Figure 3-5a, GLMM 2-1a in Table 3-4). Infant age was negatively related with the number of copulations – males copulated more with the females with younger infants (Figure 3-5b, GLMM 2-1a). Male rank was positively related with the number of copulations (high ranking males did more), and male age was negatively related with the number of copulations (Figure 3-5c, 3-5d, GLMM 2-1a). Female age did not predict the number of copulation. The interaction between infant age and days from ovulation in was not significant the model. GLMM 2-1b (Table 3-4), using all copulations from all observation days (570 copulations in 250 days), showed that male age was negatively correlated with the number of copulations. However, male rank failed to predict the number of copulations.

The number of interventions of copulation between males increased when the ovulation day was getting close (Figure 3-6a, GLMM 2-2 in Table 3-4) and increased for females with older infants (Figure 3-6b, GLMM 2-2). The number of interventions was negatively related with female age (Figure 3-6c, GLMM 2-2). As expected, high ranking males intervened more frequently in other males' copulation attempts (Figure 3-6d, GLMM 2-2). It is probably easier for high-ranking males to intervene than it is for other males. Male age was not related with the number of interventions of copulation (GLMM 2-2). There was no significant interaction between infant age and days from ovulation in this model.

Males solicited copulation more often when the day of ovulation of a given female was getting close (Figure 3-7a, GLMM 2-3 in Table 3-4). Infant age and female age did not predict the number of solicitations of copulation by males (GLMM 2-3). Both younger males (Figure 3-7b, GLMM 2-3) and high ranking males (Figure 3-7c, GLMM 2-3) solicited copulation more frequently. There was no significant interaction between infant age and days from ovulation in the model.

The number of male aggressions on a given day increased on days when male intensive following was observed (Figure 3-8a, GLMM 2-4 in Table 3-4). The number of male aggressions was also positively correlated with the number of males in the daily ranging party (Figure 3-8b, GLMM 2-4 in Table 3-4). The number of females with maximal swelling did not predict the number of aggressions between males (GLMM 2-4 in Table 3-4).

Day specific ovulation probability and fertility

The day specific probability of ovulation and fertility were calculated with 14 ovulatory cycles from 5 females. The day specific ovulation probability distributed from the 8th to 27th days from the onset of maximal swelling (Figure 3-9a). The peak probability of ovulation was on the 19th day from the onset maximal swelling. This pattern is similar to that of the previous report from Luikotale (Douglas et al. 2016), although in the present study, no ovulation was found before the onset of maximal swelling. The day specific probability of fertility – the probability that the egg coincides with sperm – did not exceed 0.3 (Figure 3-9a), which also resembles the previous finding (Douglas et al. 2016).

3.5 Discussion

In this study, I demonstrated that male mating efforts were skewed toward females with older infants and whose maximal swelling phase was close to detumescence (higher

reproductive quality). Males, regardless of rank, intensively followed females with higher reproductive quality. More specifically, the number of males in intensive following increased for females with older infants and whose maximal swelling was close to detumescence, and the interaction between infant age and days to detumescence was significant in GLMM 1-2, 1-4. This significant interaction makes sense since males do not follow females with maximal swelling when their infants are young (less than 2 years old). It is noteworthy that when the data set was restricted to the 14 ovulation cycles, the interaction disappeared (GLMM 1-3a, b). This non-significant interaction between days to ovulation and infant age indicates that male bonobos might be able to precisely determine a female's ovulation regardless of infant age. However, this could also be a sampling bias because in the reduced data set, which only contained 14 ovulatory cycles from 5 females, the variation in infant age was from 2 to 5.5 (not from 0 to 5.5).

High ranking males copulated more frequently with females with older infants and whose ovulation date was close (high reproductive quality). High ranking males also intervened in the copulation attempts of other males, when the other males tried to copulate with females with high reproductive quality. They also solicited copulation more often from females whose ovulation was close. However, solicitation of copulation was not related with infant age of females. On the days when male intensive following was observed, there were more aggressions between males.

Overall, these results support the "males know" hypothesis. The main two predictions of this hypothesis regarding male mating efforts (1-a; intensive following, 1-b; copulations, interventions and solicitations of copulation, 2; agonistic interaction) were supported by the results. Male mating efforts increase with increased ovulation probability and infant age of females. Only the result of solicitation of copulation did not support the prediction (1-b). Overall, these results suggest that bonobo males adjust their mating behaviors in relation to

female sexual swelling, as found in other species where females have exaggerated sexual swelling, including chimpanzees and Old World monkeys (Bercovitch 1988; Deschner et al. 2004; Gesquiere et al. 2007). I conclude, therefore, that male bonobos can determine the fertile phase of females based on changes in sexual skin swelling and that the sexual swelling of females is a reliable indicator of ovulation for male decision making on mating effort allocation.

Male intensive following behavior after females

Males intensively followed females with maximal swelling more than females at other stages in the sexual swelling phase. In particular, when the female had an older infant and the sexual swelling was near to the detumescence (higher reproductive quality), a higher number of males intensively followed the female (GLMM 1-1 to 1-2 in Table 3-3). Therefore, when there are several females with maximal swelling, males appear to be able to distinguish the reproductive quality of females and will selectively follow females with high reproductive quality.

GLMM 1-4 (Table 3-3) showed that the number of males following females increased over time after the onset of sexual swelling. However, caution is needed to interpret the effect of onset of sexual swelling on intensive following behaviors, because males did not follow females who had an excessively long maximal swelling phase (Figure 3-4). Males seem not to be interested much in the sexual swelling of females with young infants (younger than 2 years). Females with such young infants also had excessively long maximal swelling phase (Chapter 2). Given that males are more likely to follow females with maximal swelling and weaned infants (e.g. over 4 years old offspring), the influence of the onset of maximal sexual swelling on male decision making might be limited. However, the onset could also be

important, when there are several females with similar reproductive quality – based on reproductive history such as parities, lactation status – on a given day.

It is possible that female bonobos have some degree of synchrony of menstrual cycle, and therefore also the maximal swelling phase. Here, some degree of synchrony is not a strict synchrony in which the ovulation date of each female coincides within a narrow window of a few days (e.g. overlapping of 4 days of fertile window). Although synchrony of the menstrual cycle has not been demonstrated in bonobos, and is arguable in other species including humans (Harris and Vitzthum 2013), the birth dates of infants might have synchronized in the study group (E1). We had 10 newborn infants from 2010 to 2015 (all of them have survived), and for 6/10 births two births occurred within two weeks on three occasions, which might have resulted from some degree of synchrony of menstrual cycles. If synchrony of menstrual cycle (sexual swelling) did exist in bonobos, it would be difficult for high ranking males to monopolize copulation attempts against other males. Some degree of synchrony can also allow females to test the physical durability of males and reduce female–female competition. This looser synchrony in menstrual cycles (or swelling cycles), therefore, can play a counter strategy against the male mating strategy and allow females to exert control on it. This looser synchrony of menstrual cycles could have some regulative role in male–male competition. However, a narrow synchrony window (e.g. fertile phase; within 4 days) can result in a competitive situation between females and so is unlikely beneficial.

There was some discrepancy among the models in the influence of one predictor variable: the number of females with maximal swelling (GLMM 1-3a vs 1-3b, GLMM 1-4a vs 1-4b). This variable was added to control for temporal changes in availability of females, which might have influenced the models. Therefore, the discrepancy of the direction of prediction should not cause a big problem given that the effects of infant age, probability of

ovulation (GLMM 1-3a, b) and sexual swelling (GLMM 1-4a, b) on the models did not change.

It was also confirmed that more males intensively followed the female whose ovulation was about to occur. The way of determining ovulation was strict; ovulation was not determined unless the average of PdG was above the threshold (mean + 2SD) for 10 days after the ovulation day. It is therefore possible that I missed some ovulations, in particular from females with young infants (younger than 2 years), as was found in a previous study (Douglas et al. 2016). However, ovulation in those females might not have much influence on male behavior since males were not interested in females with such young infants, even when the females had maximal swelling (GLMM 1-1 to 1-4).

As shown in the figure 3-2a, the number of males intensively following a certain female seemed to decrease one or two days before detumescence. This might reflect that male bonobos are better than human observers in evaluating subtle changes in sexual swelling. Or it is possible that males have some ability to detect ovulation based on olfactory and other visual cues (Rigaill et al. 2013). However, such discussion goes beyond the scope of this study. Given that detumescence of maximal swelling was a good predictor of male responses, it is likely that male bonobos also rely on the sexual swelling as an important indicator of ovulation just like males of other primate species [Old World monkeys (Gesquiere et al. 2007; Young et al. 2013) and chimpanzees (Deschner et al. 2004; Thompson 2005)]. A more important question might then be whether the seemingly unpredictable ovulation of female bonobos, possibly concealed by the maximal swelling phase (Douglas et al. 2016; Street et al. 2016), would be manipulative enough to confuse male mating decision making and to regulate male–male mating competition.

Male intensive following behavior was not directed toward females whose infants were less than 2 years old, except for 2 days during Nv's maximal swelling phase and that was

only by 2 males (when her infant was 21 months old). Male intensive following sometimes continued all day, from the bed site in the morning to dusk when group members started making their beds for the night. In those cases, males would even make beds around the target female. This might be because males do not want males other than themselves sneaking copulations during the night or early next morning. All-day-long male intensive following was most noticeable when the sexual swelling of females with old infants (over 4) reached its maximal swelling phase. This might be related to the inter-birth interval, which was 4.8 ± 0.9 years in this study group (Chapter 2). In future, it will therefore be valuable to test whether or not the duration of intensive following of a given male is related with the female's reproductive quality (ovulation and infant age). It is possible that females test the durability of males by encouraging them to participate in all-day-long intensive following for better results in mate choice. Together with the possibility of loose synchrony of ovulation (e.g. ovulations from two female coinciding within one or two weeks), this female mating strategy might be a challenge, particularly for the highest ranking male who would have to invest more energy for longer periods than lower ranking males.

In all models (GLMM 1-1 to 1-4), except GLMM 1-4a, the number of males that day did not explain the increased number of males intensively following females. This indicates that the spatio-temporal distribution of males each day was not responsible for the increase in the number of males intensively following a given female. This strengthens the finding that male bonobos selectively choose the target of intensive following rather than just following any female with maximal swelling.

Male mating-related behaviors

As predicted from the "males know" hypothesis, the number of copulations within dyads increased as ovulation days got closer. However, infant age had a negative influence on the

number of copulations within dyads, which is opposite from one of the predictions of the hypothesis. It is possible that females with older infants are more selective for their choice of copulation partners, or that mate guarding from high ranking males is more intense for females with older infants so they cannot copulate so easily.

Male rank was also positively correlated with the number of copulations. High ranking males might be chosen more often by females for copulation. High ranking males can also increase their chance of copulation by staying closer to the female (Furuichi 2011; Surbeck et al. 2012). Occupying spatio-temporal proximity with a female with high reproductive quality (ovulating females with older infants) can be an effective strategy of male bonobos, given the high possibility of female choice in bonobos because of the high social status of females (Furuichi 1997; Furuichi 2011; Tokuyama and Furuichi 2016). A GLMM model using the proximity data from the individual focal sampling confirmed that the number of females with maximal swelling within 1 meter was negatively related with male age ($z = -3.41$, $se = 0.03$, $p = 0.0007$), but not related with male rank ($z = -0.15$, $se = 0.09$, $p = 0.88$). This result suggests that it is not always the high ranking males who are closer to more females with maximal swelling and their access to copulation was not simply explained by the availability of females with maximal swelling in close proximity. The result from the GLMM is consistent with the results from GLMM 2-1b (Table 3-4) that younger males copulated more frequently. High frequency of copulations in younger males (Table 3-1b) therefore, might be due to the close spatio-temporal relationship with females. Young males might get extra benefit from having their mother in the group, since the mother can influence the availability of females around their son directly and indirectly (Surbeck et al. 2011; Furuichi 2011; Ryu et al. 2015).

In this study, I did not conduct individual level analysis for copulations, as had been done previously (Deschner et al. 2004), since it was not free from the multiple comparison problem. However, the number of copulations was explained by male rank in GLMM 2-1a

(copulations only with ovulating females), but not in GLMM 2-1b (with all observed copulations). If high ranking males do not always occupy the highest proportion of copulations, we can expect that high ranking males copulate with females more selectively based on ovulation probability and infant age (reproductive quality) of the females. If that is the case, there should be a difference in copulation patterns depending upon male rank. To test this possibility, I divided the data set of GLMM 2-1a into two data sets and ran two GLMMs (GLMM 3-1 and 3-2 in Table 3-5) using the same variables in GLMM 2-1.

The first data set for GLMM 3-1 (Table 3-5) comprised the copulations of the top 3 ranking males (JR, NB, KT in periods 3 and KT, NB, GC in period 3) who occupied 78.9% of all copulations (165 in 209 copulations with females in ovulatory cycles, -14 to 0 days from ovulation). The top 3 ranking males also maintained clear-cut dominance over others during all study periods. The second data set for GLMM 3-2 comprised the other 7 males in the 4th to 10th rank in the group (Table 3-5).

These two separated GLMMs showed two important contrasts. One is that the ovulation probability was responsible for the increased number of copulations for the top 3 ranking males but not for low ranking males. The other is that infant age did not predict the number of copulations for the 3 top ranking males but it was negatively related to the number of copulations of low ranking males, which probably means that the low ranking males have a hard time to access females with higher reproductive quality. These results suggest that even though copulation in bonobos seems to be open for low ranking individuals, there is mating competition among males and the mating competition results in a skewed number of copulations toward high ranking males with highly fertile females. This result can also be explained by two different ways. One is that low ranking males are likely to be excluded by male–male competition. Therefore, they might seek access to females with lower reproductive quality to maximize chances of copulation, as well as reproductive success. If

that is the case, the extended receptivity of female bonobo, because of frequent and prolonged maximal swelling, might indeed play a regulative role in male mating competition as predicted in the estrous sex ratio hypothesis (Furuichi 2011). Another possibility is that this pattern just reflects female choice. With the growth of her infant, reproductive quality of a female will also increase. Then, the female becomes more selective when choosing mating partners. It is possible that females also prefer high ranking males to low ranking males. This could also explain the pattern found in GLMM 3-1 and 3-2.

It is also important to note that paternity in bonobos seems skewed for high ranking males (Hohmann et al. 1999) as found in chimpanzees (Boesch et al. 2006). In the chimpanzee studies, it was found that male social rank is important to predict the reproductive success, based on the number of sired offspring (Constable et al. 2001; Inoue et al. 2008; Newton-Fisher et al. 2010). Together with the previous finding that male rank is important in bonobos for mating success (Surbeck et al. 2011), it is likely that male–male competition is also an important characteristic of bonobo society. Moreover, male–male competition may cause reproductive skew toward high ranking males who are more durable and can monopolize mating with females with higher reproductive quality.

The results from the models (GLMM 2-2 and 2-3 in Table 3-4) which examined the number of interventions and solicitations of copulation are also in line with the results from the copulation model (GLMM 2-1a in Table 3-4). When a female was about to ovulate, the number of interventions by high ranking males increased. Males also intervened in copulation attempts of other males more often when the other males were trying to copulate with females with older infants. It is important to note that they raised the intervention rate for young females. This might be because old females are usually high ranking (Furuichi 1997; Tokuyama and Furuichi 2016) and their high social status might hinder males from intervening in copulations. Since we have no comparative data with chimpanzees, it is

difficult to directly compare with the rate of intervention of copulation in chimpanzees and evaluate the degree of male–male competition. However, we can say that aggressions caused by mating competition between male bonobos do not result in very severe injuries. This might be one of the biggest differences compared with chimpanzees, with chimpanzee males sometimes being involved in lethal aggression (Wrangham 1999; Wilson et al. 2014).

The solicitation of copulation was not related to infant age. This is different from the prediction (1-b). It is possible that males, particularly low ranking or/and young males, solicited copulations from females with young infants as often as from females with older infant to increase the chance of mating. Mating with females with lower reproductive quality might just be for pleasure or that females with younger infants are more likely to respond to male solicitation, so males solicited copulation from them as often as from females with older infants.

In GLMM 2-4, it was confirmed that the number of total male aggressions on a given day increased when male intensive following behavior was observed. This would strengthen the assumption that male intensive following behavior is a mating effort to attain reproductive success, rather than just watching and following a female with maximal swelling. As seen in GLMM 2-4, it is possible that the increase in male aggressions was simply due to the increase in number of males in the daily ranging party when there were males engaged in intensive following after females. However, using the same data set of GLMM 2-4, I confirmed that the number of males in the daily ranging party was not different in relation to the existence of male intensive following (GLMM, $z = 1.17$, $se = 0.05$, $p = 0.24$). Therefore, it is likely that male mating competition results in the increased number of aggressions in the daily ranging party of bonobos. We did not find any influence of the number females in maximal swelling in GLMM 2-4. However, when the number of males on a given day was controlled in GLMM 2-4 using offset term, the number of females with maximal swelling was positively related

with the number of male-male aggression (GLMM, z= 3.43, se = 0.29, p = 0.0006). In this model, we used two offset terms; following hours of a given day and the number of males on the day. Altering the offset term did not seem to cause a problem, since the relationship between the predictor variables; the response variable; and the statistical significance of the model, as well as the GLMM estimations, did not change from GLMM 2-4.

Probability of ovulation on a certain day

In this study, I showed that mating efforts of male bonobos increased in intensity around ovulation. This is very similar to the finding from chimpanzees (Deschner et al. 2004). Considering that the onset and detumescence of maximal swelling were good predictors of male mating efforts (GLMM 1s and 2s), it is likely that males rely on the sexual swelling of female bonobos as a signal to make decisions for mating. However, as shown in Figure 3-9a day specific probability of ovulation and fertility (sum of 4 days of probability of ovulation) resembles the previous findings from bonobos (Street et al. 2016; Douglas et al. 2016). This indicates that the predictability of ovulation from the onset of the maximal swelling phase is also low in this study group.

However, the day specific probability variations of fertility from the onset of maximal swelling in Figure 3-9a resemble the pattern shown in figure 3-4, that more males are involved in intensive following as the days from the onset increased. This suggests that males can adjust their mating efforts in relation to the fertility of females.

It was suggested that sexual swelling of bonobos might deviate from the sexual swelling of other primate species including chimpanzees (Douglas et al. 2016). However, the physiological mechanism which results in this difference was not suggested. Together with the findings from the Chapter 2, it is likely that the basic hormonal mechanism of the regulation of sexual swelling in bonobo did not change much from that of chimpanzees and

other primates. It is also true that increase in estrogen coincides with onset of maximal swelling and constant rise of progesterone after an ovulation results in detumescence (Chapter 2; Street et al. 2016). Therefore, it seems less likely that the sexual swelling of female bonobos has evolved to be more independent from the hormonal mechanism. If the sexual swelling of bonobo becomes more independent from ovulation, why do they still keep this costly signal? It is possible that we have missed some important keys or that we are stuck with noise or artifacts from the previous model to predict ovulation probability.

The periods of maximal swelling phase for female bonobos vary among individuals and between cycles within an individual (Chapter 2; Douglas et al. 2016). This high variation could be an artifact caused by differences in methodology used to define the maximal swelling phase at different bonobo study sites and by different researchers. However, it seems that it more likely reflects the physiological mechanism that regulates the swelling size by sexual hormones, as they resume sexual swelling cycle even in early lactation periods; 240.5 days in average after giving birth (Chapter 2). This variation might be the reason (or the source of noise) why the probability of ovulation and fertility, calculated from the onset of maximal swelling using the equations defined by Deschner and his colleagues (Deschner et al. 2003), were more evenly distributed in bonobos compared to chimpanzees (see Figure 3-9a, and Douglas et al. 2016). In other words, high individual variation in the length of maximal swelling phase, could result in poor predictability of ovulation from the onset of maximal swelling. If that is the case, then poor predictability cannot be interpreted as concealing ovulation to manipulate male bonobos. Another weak part of this equation is that it does not take into account variation in the length of the follicular phase of menstrual cycles between and within individual across cycles compared to the luteal phase (Dixson 2012). The variation in the follicular phase length would inevitably cause errors between studies and might mislead researchers to draw different conclusions on the mating strategy of males in

species for which variation in the length of maximal swelling phase is great (e.g. bonobos). If ovulation is barely predictable in bonobos, how would males be able to match their mating efforts near to ovulation and detumescence of maximal swelling, as shown in this study? I suggest that the physiological mechanism regulating sexual swelling might be the first key to solve this puzzle.

A recent review showed that sexual swelling is, in general, a reliable signal indicating fertility of females (Street et al. 2016). However, that study did not provide any models to explain how males in different species cope with variations in the reliability of the signal (indicate ovulation) through their mating efforts. Bonobos provide a unique opportunity to understand male mating strategy, as bonobos are thought to have the lowest reliability of the signal among species with maximal swelling (Street et al. 2016).

To understand how male bonobos deal with the low reliability of the signal, I propose an alternative view (or a model), which calculates the distribution of ovulation probability and fertility from the day of detumescence, rather than from the onset of maximal swelling. This might also provide a useful framework for understanding how males rely on the graded-signaling of ovulation. This proposal is based on the physiological constraints of the regulatory mechanisms of sexual swelling – estrogen and progesterone work antagonistically to the sexual swelling size changes – that an increase of serum progesterone after ovulation results in detumescence of sexual swelling (Gillman 1940; Carlisle et al. 1981; Ozasa and Gould 1982). This model does not need to assume that males can pin point the detumescent day of the maximal swelling phase. Instead, they can simply expect that each day the probability of ovulation will get higher on the next day, if the detumescence does not happen on that day.

When ovulation occurred, in most cases, detumescence of the maximal swelling also coincided (Chapter 2). I calculated the day-specific probability of ovulation and the fertility

from the detumescent day (Figure 3-9b) following the same equation (Deschner et al. 2003) used for figure 3-9a. Figure 3-9b only differs from Figure 3-9a in that day 0 in the x-axis, was rearranged to the detumescent day, not the day of the onset of the maximal swelling. As expected, the probability of ovulation and fertility was very high near to the detumescent day so that copulation around detumescence would more likely result in pregnancy than copulation on other days. This might also explain the results that male mating efforts, including intensive following and aggressions, were more concentrated near to ovulations and detumescence and that these behaviors decreased just before detumescence as in Figure 3-2a and 3-2c.

I also predict that there will be less inter-species variation (among species with sexual swelling) in the probability of ovulation from the detumescent day. Holding this assumption, I propose a model of male response toward the manipulative ovulation signal from sexual swelling of female bonobos. This model, however, would be also applicable to understanding male mating effort allocation, not only in species where sexual swelling is found but also in species that have any graded-signals indicating ovulation (e.g. changes in face colors, odors, behaviors in relation to ovulation probability). The model assumes that male bonobos, as well as males in the species proposed above, might adopt simple rules to allocate their mating efforts for the best reproductive outcome.

The first layer is called ‘just-do-it’. Males may seek any copulation opportunity. Whenever the opportunity for copulation is available, males are willing to engage in copulation. This layer assumes that males are highly motivated for copulation, which is plausible considering that more mating in general might give males better reproductive outcomes (Bateman 1948). In particular, this layer could be a predominant rule for young males throughout their puberty as more copulations might allow them to become better at copulation.

The second layer is that males allocate more mating efforts for access to females with older infants (called ‘check infant age’). This requires that males have some understanding of the Inter-birth interval or changes in reproductive quality based on one’s infant growth. Holding this rule by males, does not necessarily result in skewed copulations toward the females with higher reproductive quality (females with older infants and higher probability of ovulation), since male–male competition and female choice might influence the outcomes of copulation. Given that inter-birth interval of female bonobos is around 5 years, infant age is likely to be a good predictor of male mating effort allocation. It is not necessarily assumed that males should recognize infant age as humans do. It can work if the male can somehow discriminate females with different reproductive history based on some signal or cue related to one’s infant age.

The third layer might be more complicated and arguable. This layer is called ‘lottery’. If maximal swelling (or any indicative signal of ovulation) continued on the next day, then males simply increase mating efforts or keep a similar degree of mating effort toward the female on that day compared to yesterday. Males, in particular higher ranking males, might skew their mating efforts more toward females with older infants and whose maximal swelling is close to detumescence (or the onset of maximal swelling was already many days ago). This does not mean that they can perfectly predict ovulation. They can make wrong decisions, in particular when the ovulations of several females are closely synchronized. It is also plausible that males are interested in a female from the onset of her maximal swelling phase, particularly for a female accompanied by an old (or weaned) infant, and males maintain their mating interests and efforts until the day of detumescence. After detumescence, they will not pay attention, since the probability that the sexual swelling gets detumescent before ovulation are very low (though not zero). Since social-ecological factors influencing male rank in bonobos, such as the presence of mother, party size and

composition, might vary over time in males, these variations or irregularities between individuals can cause individual specificity of the third layer. Therefore, the strategy of each male for the 3rd layer might vary between individuals as well as within an individual over his lifetime.

Overall, this model explains the results of this study that male mating efforts get more intense around the time of ovulation. We call this hypothetical model a “lottery” model since it is somewhat similar to the way lotteries work. In lotteries, as the prize money increases when there are no winners for several weeks, the more people buy lottery tickets. This might increase the chance of a winner coming out in the whole game over time, though it is more related with pure probability of chance (Bercovitch, personal communication).

It is also possible that males are able to predict the fertile phase pretty precisely using multiple signals (or cues), including female behavior and olfaction. This might also explain the decrease in the number of males engaged in intensive following even before detumescence (Figure 3-2a, 3-2c, 3-4). However, there are no such data to test this possibility in this study.

It was reported that the size of sexual swelling gradually increases until the fertile phase (or peri-ovulatory periods) in chimpanzees (Emery and Whitten 2003; Deschner et al. 2004) and in olive baboons (Higham et al. 2008). Based on this finding, it was suggested that males might be able to discriminate the subtle change in the size of sexual swelling (Deschner et al. 2004). However, it is possible that increase in size of sexual swelling just reflects the gradual increase of serum estrogen level until ovulation and males do not need to discriminate well the subtle size variation within the maximal swelling phase (see also Young et al. 2013 for the case in Barbary macaques). If it is the case, the gradual increase of size within the maximal swelling phase can only have significance when it is presented to the males with continuous time scale. This possibility will be examined in future study. With more precise

measurement of female's signals, the future study will clarify whether or not males have the ability to discriminate subtle increases of size and color (or even olfaction) and adjust their mating efforts precisely to the narrow fertile window. This will provide us with a valuable opportunity to understand how males have evolved to respond to females' signaling on ovulation probability.

It is known that an increase of serum progesterone concentration caused by ovulation results in detumescence (Gillman 1940; Kato et al. 1980). There could be some variation in the exact day of detumescence between individuals and/or cycles within an individual. However, we do not know yet any mechanism standing for the physiology, which does not follow this basic mechanism or that causes considerable variations if there is. If there is a mechanism that can explain how an increase of progesterone would not result in detumescence, it would be very interesting to look deeper into the reason why. However, it is unlikely that the physiology of sexual hormones have completely changed only in bonobos. Instead, it is likely that the reproductive history of a female, including lactation, pregnancies, and miscarriages (early loss; Wilcox et al. 1988), might be related to the high variation in length of maximal swelling phase (as these events add more instability to the follicular phase) in bonobos. The physiological variations from one's reproductive history might be one of the most important sources of the noise which causes the loose relationship between the probability of ovulation and sexual swelling (Figure 3-9a, see also Chapter 2).

In this study, I reconfirmed that male–male competition also exists in bonobos, as found previously (Surbeck et al. 2011), and male mating competition is important for males to access females in the fertile phase. In addition, I found that males concentrated their mating efforts more on the females whose maximal swelling phase was close to detumescence and who were with older infants. These results suggest that male mating efforts and interests are not manipulated by the poor predictability of ovulation from the onset of the maximal

swelling. Instead, as I suggested in the three-layered model, they might be able to cope with the poor probability of ovulation and their mating efforts using some simple rules based on sexual swelling and infant age.

Chimpanzee society is characterized as male centered and aggressive. However, bonobo society is recognized as female centered and peaceful (de Waal 1989; Parish et al. 2000; Furuichi 2011; Clay et al. 2016). The prolonged receptivity from early resumption of maximal swelling phase and long maximal swelling phase (Chapter 2) of bonobos was suggested as the key to understanding the difference in social characteristics between chimpanzees and bonobos (de Waal 1989; de Waal 1995; Furuichi 2011). It was suggested because more open chances of mating even for low ranking males in bonobo society from prolonged maximal swelling was considered to lower male-mating competitions in bonobos. However, it has not yet been thoroughly tested whether more open chances of copulation for low ranking males, does indeed contribute for lowering male-mating competition in this species. It is also questionable whether the prolonged periods of maximal swelling phase actually conceals ovulation and then that concealed ovulation lowers mating competition. It is also possible that if ovulation is concealed, males might need to allocate more efforts toward mate guarding and sexual activity of females for a longer time (Burley 1979; Dixson 2012), which then might increase male-mating competition.

As found in this study, male mating efforts are more likely allocated to females with higher reproductive quality. However, it was also found that males engaged more in copulation with females with younger infants who probably have low reproductive quality. This tendency was most prevalent for lower ranking males. Therefore, it is possible that extended sexuality achieved by the more frequent and prolonged maximal swelling phase of female bonobos has some regulative role in male mating competition. Together with the possibility that females are getting more selective when their infant gets older, future studies

should examine this possibility and clarify the regulative role of sexual interactions between males and females with low reproductive quality.

The main perspective and insight from this study are that the extended periods of receptivity of female bonobos through frequent and prolonged maximal swelling phase is not enough to explain the social differences between chimpanzees and bonobos.

In contrast to female chimpanzees (Kahlenberg et al. 2008), bonobo females make coalitions against male aggression (Tokuyama and Furuichi 2016) and this might contribute to the high social status of female bonobos. The high social status of female bonobos might also result in less restricted copulation patterns, even for lower ranking males. Together with this possibility, I suggest that the sexuality and lower predictability of ovulation of bonobos might not be the only causation of the less aggressive and more tolerant bonobo society (peaceful society). Instead, the peaceful nature and the less restricted sexuality can be rather the outcome from high social status of females (Alpha female hypothesis) which is as high or equivalent to that of males. Nevertheless, the extended sexuality from the prolonged sexual swelling phase of female bonobos can have some regulative roles in male competition, although this study suggested that it might not be efficient for females to have complete control over male–male competition toward better reproductive success. Therefore, I suggest that to understand the characteristics of bonobo society, we have to consider the socio-ecological factors underpinning how females achieved the high social status in their society, as well as sexual traits of females and males.

3.6 Authors' contributions

Conceptualization, H. Ryu; Investigation, H. Ryu; Methodology, H. Ryu, K. Mouri, K. Shimizu, C. Hashimoto; Formal Analysis, H. Ryu; Writing – Original Draft, H. Ryu; Writing – Review & Editing, H. Ryu, C. Hashimoto, D.A. Hill, K. Mouri, K. Shimizu, T. Furuichi;

Project Administration, T. Furuichi; Supervision, T. Furuichi, D.A. Hill; Funding Acquisition, T. Furuichi, C. Hashimoto, H. Ryu (following Brand et al. 2015).

Table 3-1. Female and male composition of E1 group

The age in 2015 of each female was estimated based on immigration date and several morphological cues. The age of males was estimated based on their birth date or morphological cues. The month of immigration of Yk, Hs, Jk and Sl were not known.

a) Basic information of female subjects in this study. Infant age is in months.

Name	Ovulation Cycles (an)	MSP cycles	Urine samples	Age (2015)	Infant age (2015.04)	Immigration	Latest delivery
No	3 (0)	9	120	44	0	1983.11	2015.04
Ki	0 (0)	4	44	41	15	1984.12	2014.02
Yk	0 (0)	5	29	34	13	2004.04	2014.04
Hs	0 (2)	2	36	32	15	> 2003.08	2014.02
Jk	4 (0)	7	60	27	39	2004.04	2012.01
Sl	2 (0)	9	114	24	40	> 2003.08	2011.12
Nv	0 (2)	5	53	20	21	2007.08	2013.7
Ot	1 (0)	3	62	18	0	2008.06	2015.04
Fk	4 (1)	7	139	17	51	2008.06	2011.02

b) Basic information of male subjects in this study.

Name	Age (2015)	Age class	Copulation	Rank p1	Rank p2 p3	Identified (age)
TN	45	Old	21	8	8	1976 (6)
TW	41	Old	0	7	7	1976 (2)
DI	40	Old	20	9	10	2004 (29)
NB	27	Middle	68	2	2	1988 (0)
GC	27	Middle	18	4	3	2003 (15)
LB	22	Middle	18	5	6	2003 (12)
JD	22	Middle	39	6	4	2003 (12)
JR	14	Young	34	1	5	2004 (3)
KT	11	Young	274	3	1	2004 (0)
SB	11	Young	78	10	9	2004

Table 3-2. Male dyadic agonistic interactions during the study periods

In Study Period 1, JR occupied the highest rank. However, the h′ index (0.84) indicated that the hierarchy was not clearly linear. In Study Period 2 and 3 the hierarchy was clearly linear (h′ = 0.95). The numbers right to the name is the age of the individual in 2014 (a) and 2015 (b).

a) Male agonistic interaction in Study Period 1.

ID	JR	NB	KT	GC	LB	JD	TW	TN	DI	SB
JR (13)		10	13	21	9	10	1	5	2	20
NB (26)	6		0	30	4	9	0	5	1	12
KT (10)	0	5		2	8	4	0	8	2	15
GC (26)	4	2	1		10	0	2	4	1	2
LB (20)	0	0	2	0		1	5	3	7	6
JD (20)	0	0	0	0	0		3	2	7	18
TW (40)	0	0	0	1	0	0		3	0	0
TN (44)	0	0	0	0	1	0	0		8	8
DI (39)	0	0	1	0	0	0	1	0		9
SB (10)	0	0	0	0	0	0	0	0	0	

b) Male agonistic interaction in Study Period 2 and 3.

ID	KT	NB	GC	JD	JR	LB	TW	TN	SB	DI
KT (11)		40	15	6	20	26	1	3	28	4
NB (27)	2		31	8	56	19	3	7	8	4
GC (27)	0	0		1	84	6	2	4	10	9
JD (21)	0	0	0		0	11	5	5	9	9
JR (14)	3	8	0	0		1	0	1	2	2
LB (21)	0	0	0	0	0		8	7	4	25
TW (41)	0	0	0	0	0	0		2	0	1
TN (45)	0	0	0	0	0	0	0		1	8
SB (11)	2	0	0	0	0	0	0	0		0
DI (40)	0	0	0	0	0	0	0	0	3	

Table 3-3. GLMM results of male intensive following (1-1 to 1-4)

Negative binomial (1-1 to 1-3) and binomial (1-4) GLMM results of the number of males who intensively followed a given female in relation to sexual swelling, infant age, the number of males, number of females with maximal swelling, days from detumescence, and ovulation.

Models	predictors	estimates	se	z	p
GLMM 1-1	intercept	-14.883	1.751648	-8.49657	1.95E-17
	infant age[1]	10.10854	1.896617	5.329772	9.83E-08
	swelling score[1]	6.308896	0.848078	7.439048	1.01E-13
	female age	-0.06582	1.829615	-0.03597	0.971303
	no. males	-0.78447	0.775792	-1.01118	0.311928
	no. FMS	-0.14395	0.783683	-0.18369	0.854258
GLMM 1-2*	Intercept	-18.1157	2.054642	-8.81694	1.18E-18
	infant age[1]	9.410117	1.810755	5.196792	2.03E-07
	days from detum.[1]	13.81645	1.86244	7.418468	1.18E-13
	female age	-0.35285	1.828525	-0.19297	0.846982
	no. males	-0.26843	0.717343	-0.3742	0.708255
	no. FMS	-0.65844	0.626621	-1.05078	0.293361
GLMM 1-3a	Intercept	-11.7139	1.243054	-9.42345	4.36E-21
	infant age[1]	5.471368	1.26292	4.332315	1.48E-05
	days from ovul.[1]	8.589316	0.965711	8.894289	5.88E-19
	female age	-0.77524	0.949632	-0.81636	0.414296
	no. males	0.723096	0.589939	1.225712	0.220307
	no. FMS	0.416246	0.523544	0.795055	0.426581
GLMM 1-3b	Intercept	-10.9604	1.393689	-7.86434	3.71E-15
	infant age[1]	5.314068	1.250267	4.250346	2.13E-05
	days from ovul.[1]	7.465575	1.272093	5.868733	4.39E-09
	female age	-0.78282	0.918262	-0.8525	0.393934
	no. males	0.259649	0.669805	0.387649	0.698275
	no. FMS	1.41477	0.596475	2.371886	0.017698

Models	predictors	estimates	se	z	p
GLMM 1-4a*	intercept	-7.95175	1.073161	-7.40965	1.27E-13
	infant age[1]	10.28846	1.219433	8.437084	3.25E-17
	days from onset[1]	4.294947	0.545115	7.878972	3.30E-15
	female age	-0.12525	1.743957	-0.07182	0.942747
	no. males	-1.95498	0.419139	-4.66428	3.10E-06
	no. FMS	-1.17046	0.417045	-2.80656	0.005007
GLMM 1-4b* result from 100 time simulations (mean ± sd)	intercept	-8.40 ± 2.30	1.43 ± 0.23	-5.84 ± 1.07	2.2E-6 ± 7.6E-6
	infant age[1]	10.37 ± 2.45	1.69 ± 0.25	6.12 ± 1.00	5.0E-7 ± 2.0E-6
	days from onset[1]	5.01 ± 2.39	0.89 ± 0.25	5.4 ± 1.11	3.6E-5 ± 1.3E-4
	female age	-1.90 ± 0.95	0.65 ± 0.06	-2.97 ± 1.47	0.09 ± 0.19
	no. males	0.05 ± 0.68	1.80 ± 0.71	-0.02 ± 0.47	0.75 ± 0.19
	no. FMS	-1.34 ± 0.99	0.63 ± 0.08	-2.03 ± 1.41	0.16 ± 0.24

All predictor variables were re-scaled 0 (minimum) to 1 (maximum). The coefficients of each model did not include interaction terms.

* The model with significant interactions found ($p<0.05$).

[1] Interaction between two variables included in each model, detum.: Detumescence, ovul.: Ovulation.

Table 3-4. GLMM results of copulation related behaviors (2-1 to 2-4)

The number of copulations (GLMM 2-1a), interventions (GLMM 2-2) and solicitations (GLMM 2-3) of copulation per hour by males between male-female dyads in relation to days from ovulation (ordinal; -14 to 0), infant age, female age (parity), male age and rank (ordinal; 1 to 10) from 48 male-female dyads in 104 days in the models. In GLMM 2-1b, and GLMM 2-4, all days of observation (250 days) were used to fit the model (see methods).

Models	predictors	estimates	se	z	p
GLMM 2-1a. copulation (-14 to 0)	intercept	-3.08044	0.782452	-3.93691	8.25E-05
	days from ovul.	2.351313	0.931336	2.524666	0.011581
	infant age	-1.45424	0.554192	-2.62408	0.008688
	female age	0.288389	0.478906	0.602184	0.547052
	male age	-3.62853	0.635652	-5.70836	1.14E-08
	male rank	-1.3506	0.470577	-2.8701	0.004103
GLMM 2-1b. all copulations	intercept	-2.37708	0.492151	-4.82997	1.37E-06
	male age	-2.41884	0.830959	-2.9109	0.003604
	male rank	0.14771	0.500856	0.294915	0.768059
GLMM 2-2. intervention (-14 to 0)	intercept	-6.419	0.977007	-6.57006	5.03E-11
	days from ovul.	2.796468	0.815436	3.429414	0.000605
	infant age	2.954038	1.246778	2.369338	0.01782
	female age	-1.27319	0.629103	-2.02382	0.042989
	male age	-1.62616	1.112004	-1.46237	0.143641
	male rank	-6.47961	1.670208	-3.87952	0.000105
GLMM 2-3. solicitation (-14 to 0)	intercept	-2.55547	0.426245	-5.99531	2.03E-09
	days from ovul.	1.008402	0.333472	3.023949	0.002495
	infant age	-0.53197	0.558792	-0.95201	0.341094
	female age	0.635899	0.409332	1.553502	0.120303
	male age	-3.77774	0.591723	-6.3843	1.72E-10
	male rank	-0.97219	0.41985	-2.31558	0.020581

Models	predictors	estimates	se	z	p
GLMM 2-4.	intercept	-2.45954	0.233404	-10.5377	5.79E-26
All aggressions	intense.fl (x: o)	-0.64606	0.112715	-5.7318	9.94E-09
	no. FMS	0.12162	0.333167	0.365042	0.71508
	no. males	2.837466	0.32358	8.768975	1.80E-18

All predictor variables were re-scaled 0 (minimum) to 1 (maximum). ovul.: ovulation, Intense.fl (x: o); male's intensive following observed or not.

Table 3-5. GLMM results of male rank and copulation (3-1 to 3-2)

The number of copulations of the top 3 ranking males (GLMM 3-1) and of the other 7 low ranking males (GLMM 3-2) in relation to the predictor variables in the models. The variables are the same in GLMM 2-1 in Table 4.

Models	predictors	estimates	se	z	p
GLMM 3-1. Top 3 ranking males	intercept	-2.21367	0.396921	-5.5771	2.45E-08
	days from ovul.	1.255983	0.398558	3.151317	0.001625
	infant age	0.129552	0.511699	0.253179	0.80013
	female age	-0.88311	0.367667	-2.40192	0.016309
	male age	-2.23023	0.302358	-7.37612	1.63E-13
	male rank	0.209644	0.297747	0.704099	0.481371
GLMM 3-2. Low ranking 7 males	intercept	-2.21367	0.396921	-5.5771	2.45E-08
	days from ovul.	0.105778	0.575434	0.183823	0.854152
	infant age	-2.04343	0.611889	-3.33954	0.000839
	female age	0.950406	0.564459	1.683746	0.092231
	male age	-2.94846	0.619863	-4.75663	1.97E-06
	male rank	0.901997	0.51649	1.746399	0.080742
	male rank	0.901997	0.51649	1.746399	0.080742

Figure 3-1. The number of males in intensive following across all days

Box-and-whisker plots, which represent the number of males on a given day who intensively followed females in relation to sexual swelling score and infant age. The number of males intensively following females was divided by the amount of following hours for each day, because the daily observation hours varied each day (Figure 3 as well). The dots represent the data point of each day.

a) The number of males who intensively followed females on a given day in relation to sexual swelling phase (GLMM 1-1). 1 is non-swelling, 2 is intermediate swelling, 3 is the maximal swelling.

b) The number of males who intensively followed females on a given day in relation to infant age of females (GLMM 1-1). Infant age ranges -1 to 5 and the minus value represents pregnant periods which included post conception swelling and the maximal swelling after ovulation which resulted in successful pregnancy. Infant age 0 is from the birth to within 1year, and infant age 1 is from 1 to <2 years and so on.

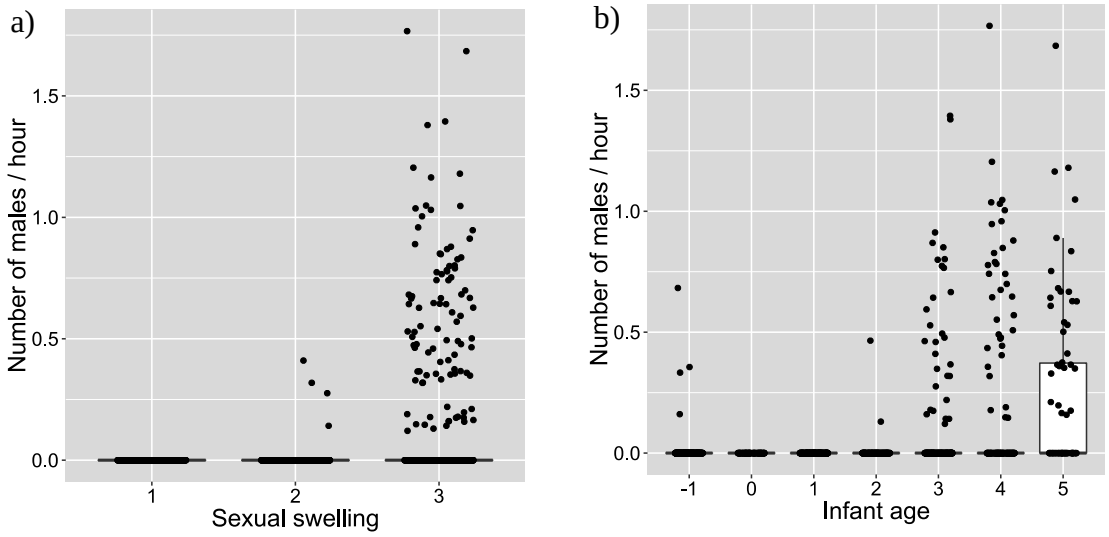


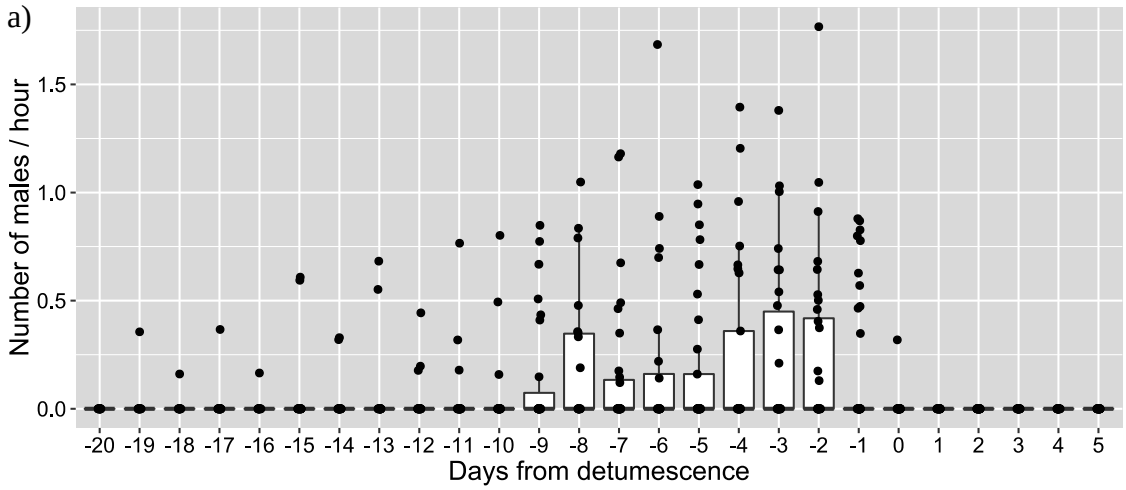
Figure 3-2. Male intensive following of females with maximal swelling

Box-and-whisker plots (3-2a, 3-2b), which represent the number of males each day who intensively followed females in relation to infant age and days from detumescence, only for females with maximal swelling (205 days in 9 females, 51 maximal swelling cycles). The dots represent the data point for each day.

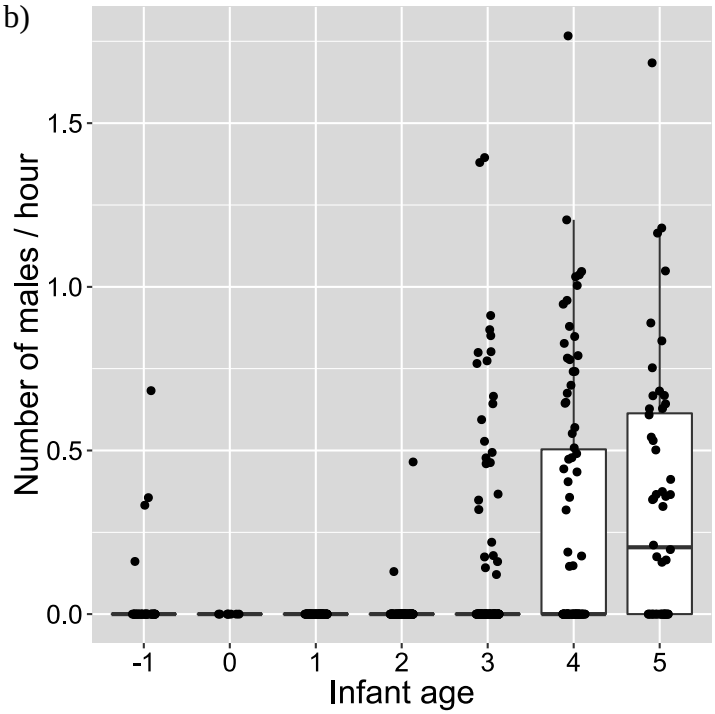
a) The number of males who intensively followed females on a given day in relation to the days from detumescence (GLMM 1-2). The day 0 is the day that the maximal swelling of a given female became detumescent.

b) The number of males who intensively followed females on a given day in relation to infant age of the females (GLMM 1-2).

c) The conditional influence between infant age of females and the detumescence day (interaction term in GLMM 1-2). This shows how the number of males in intensive following per hour (y-axis in the 4 scatter plots) increases with infant age (the age categories in the top pane) and days from detumescence (x-axis in the 4 scatter plots).



b)


Number of males / hour
0.0
0.5
1.0
1.5
-1
0
1
2
3
4
5
Infant age

c)

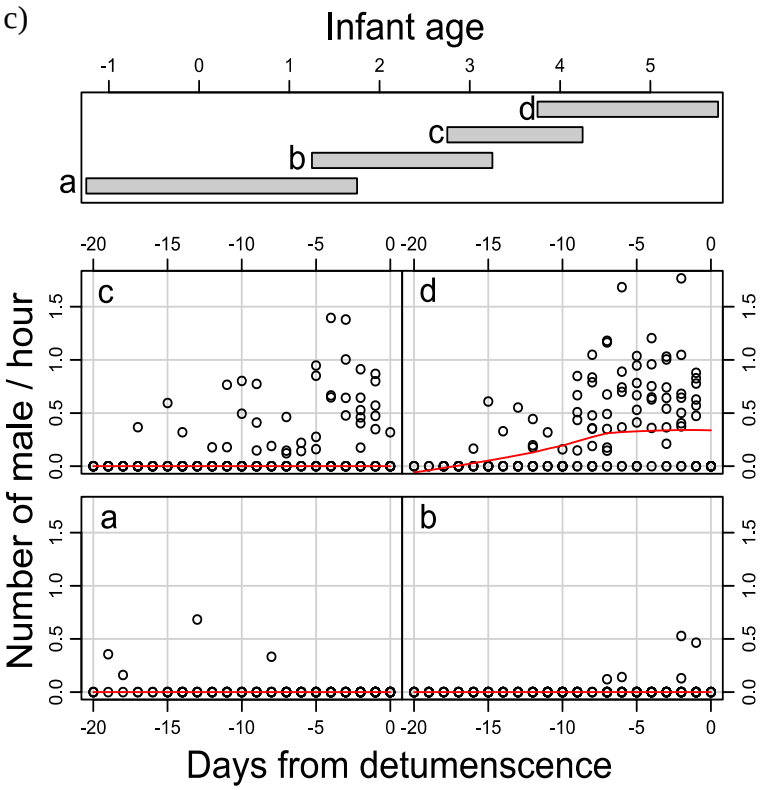

Infant age
-1
0
1
2
3
4
5
a
b
c
d
-20
-15
-10
-5
0
Number of male / hour
0.0
0.5
1.0
1.5
Days from detumenscence

Figure 3-3. Male intensive following in relation to ovulation

Box-and-whisker plots, which represent the number of males each day who intensively followed females in relation to the days from ovulation from 14 cycles from 5 females. The number of males who intensively followed females on a given day increased when ovulation was close (GLMM 1-3a). The day 0 is the day that ovulation occurred. Detumescence of maximal swelling started after 1.9 ± 2.4 days from ovulation day. The median number of males in intensive following of a given female was highest on the day of ovulation.

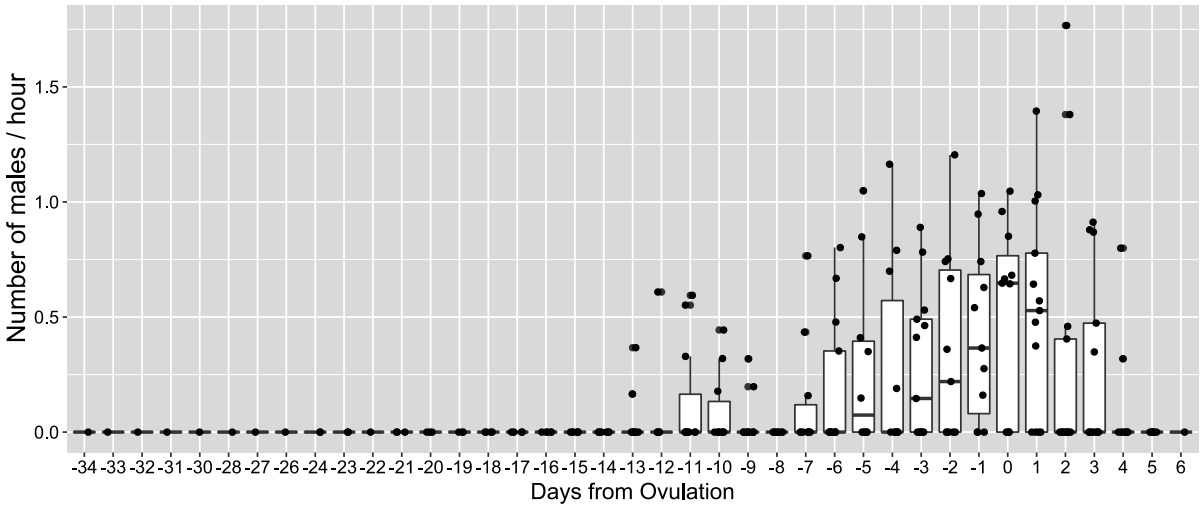


Figure 3-4. Male intensive following in relation to the onset of maximal swelling

Box-and-whisker plots, which represent the number of males each day who intensively followed females in relation to days from the onset of maximal swelling (GLMM 1-4a). The day 0 is the day that maximal swelling phase started.

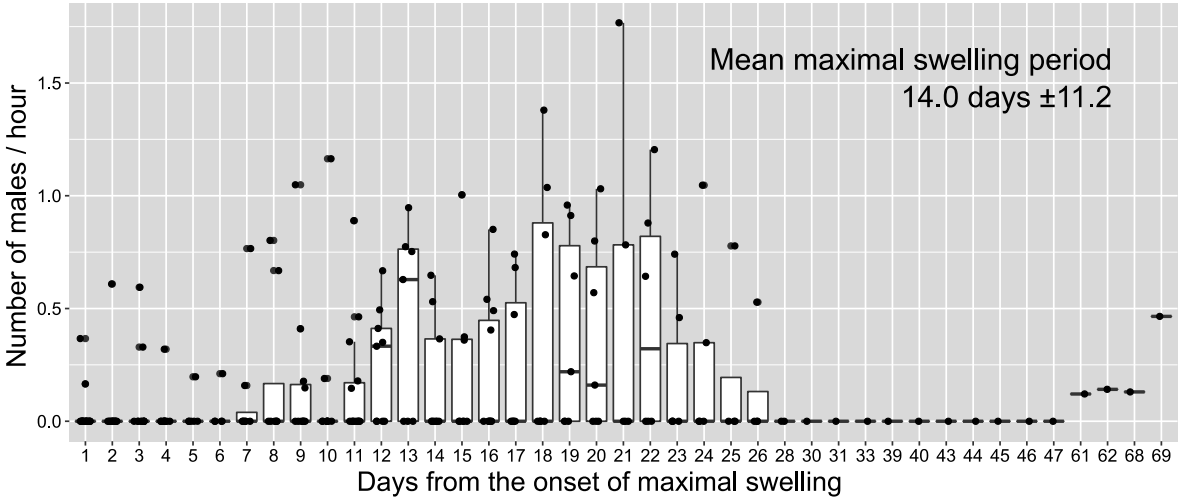


Figure 3-5. Prediction plots of males' copulations

The prediction plot of the number of copulations of males in relation to the days from ovulation, infant age, male age, and male rank (GLMM 2-1 in Table 3-4).

a) The number of copulations in relation to ovulation day (GLMM 2-1).

b) The number of copulations in relation to infant age (GLMM 2-1).

c) The number of copulations in relation to male age (GLMM 2-1).

d) The number of copulations in relation to male rank (GLMM 2-1).

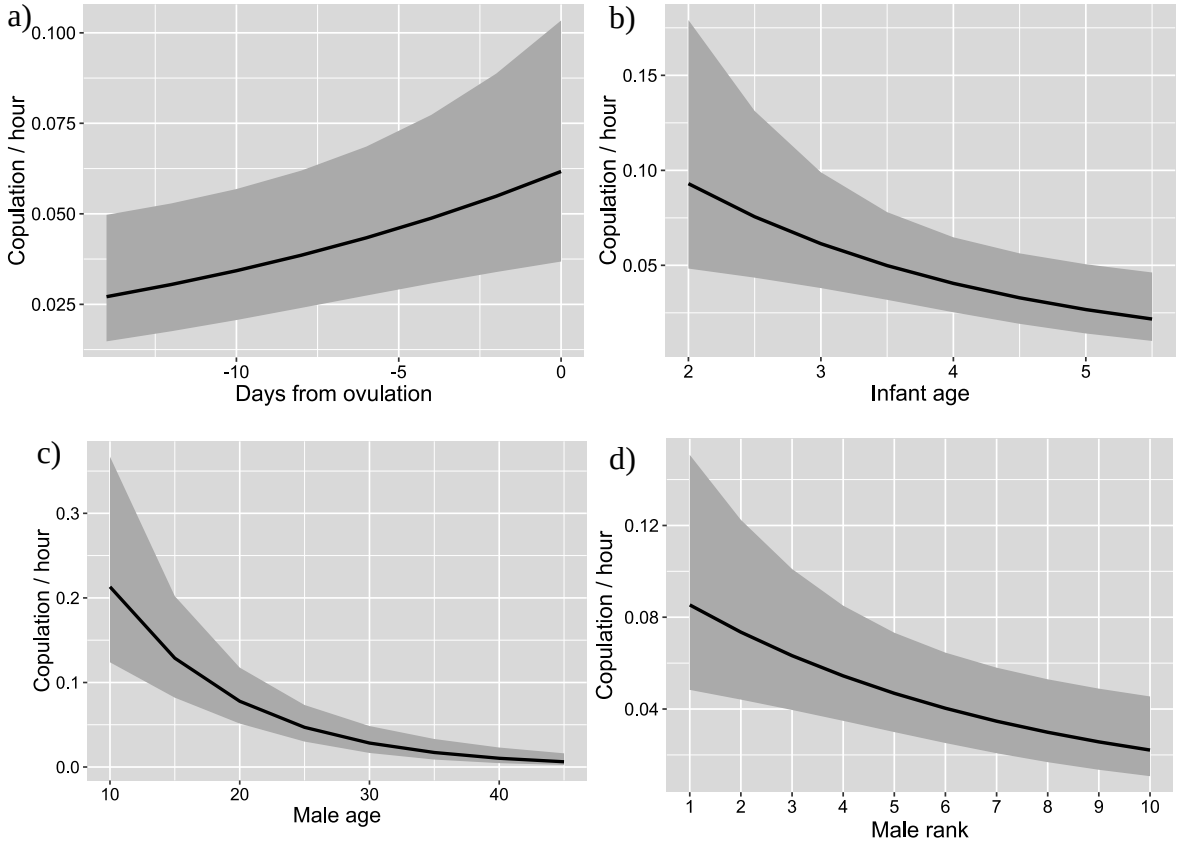


Figure 3-6. Prediction plots of intervention of copulation by males

The prediction plot of the number of males' interventions in copulation of other males in relation to the days from ovulation, infant age, female age, and male rank (GLMM 2-2 in Table 3-4).

a) The number of interventions in relation to ovulation day.

b) The number of interventions in relation to infant age.

c) The number of interventions in relation to female age.

d) The number of interventions in relation to male rank.

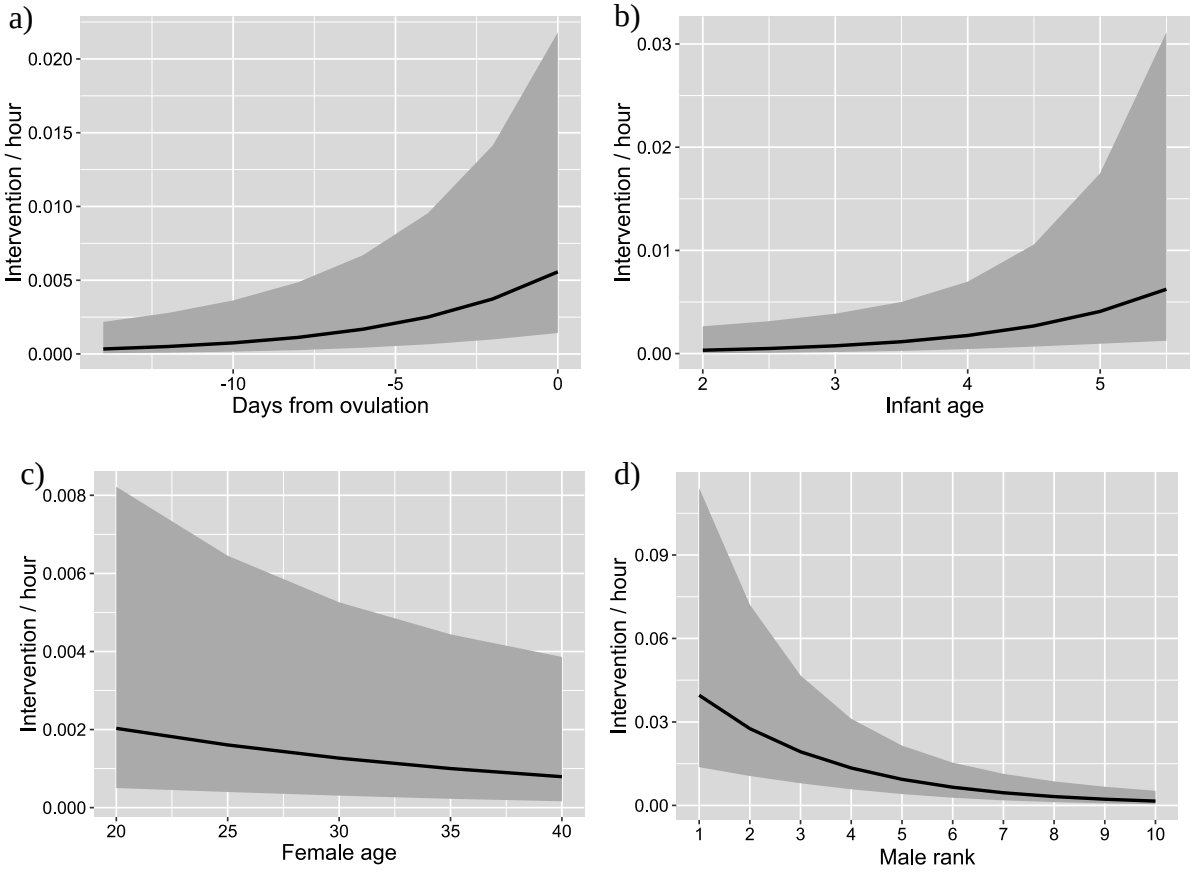


Figure 3-7. Prediction plots of solicitation of copulation by males

The prediction plot of the number of solicitations of copulation by males in relation to the days from ovulation, male age, and male rank (GLMM 2-3 in Table 3-4).

a) The number of solicitations in relation to ovulation day.

b) The number of solicitations in relation to male age.

c) The number of solicitations in relation to male rank.

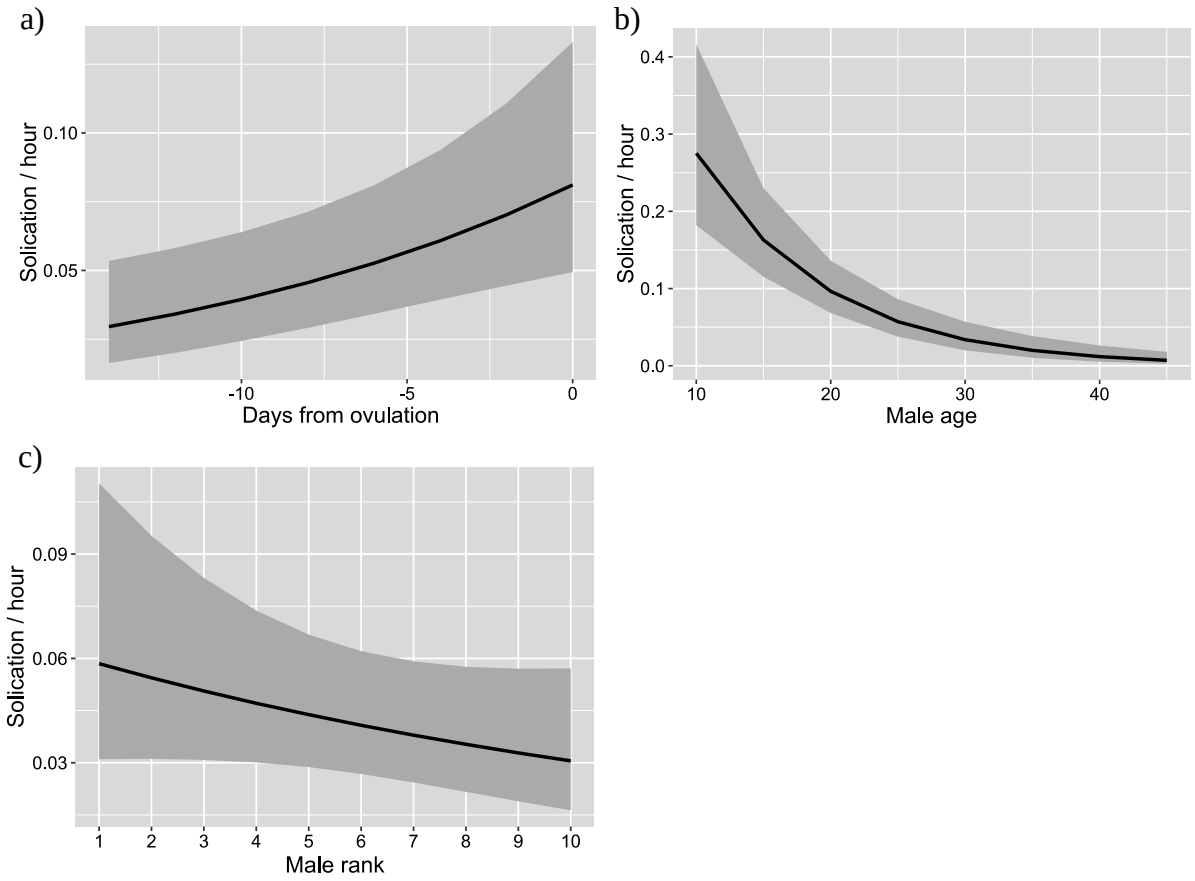


Figure 3-8. Number of male-male aggressions per day

Box-and-whisker plots, which represent the number of total aggressions each day between males in relation to the existence of male intensive following behavior on females, the number of males, and the number of females with maximal swelling.

a) Difference in the number of aggressions between males in relation to the existence of male intensive following (GLMM 2-4).

b) The number of aggressions in relation to the number of males in the daily ranging party (GLMM 2-4).

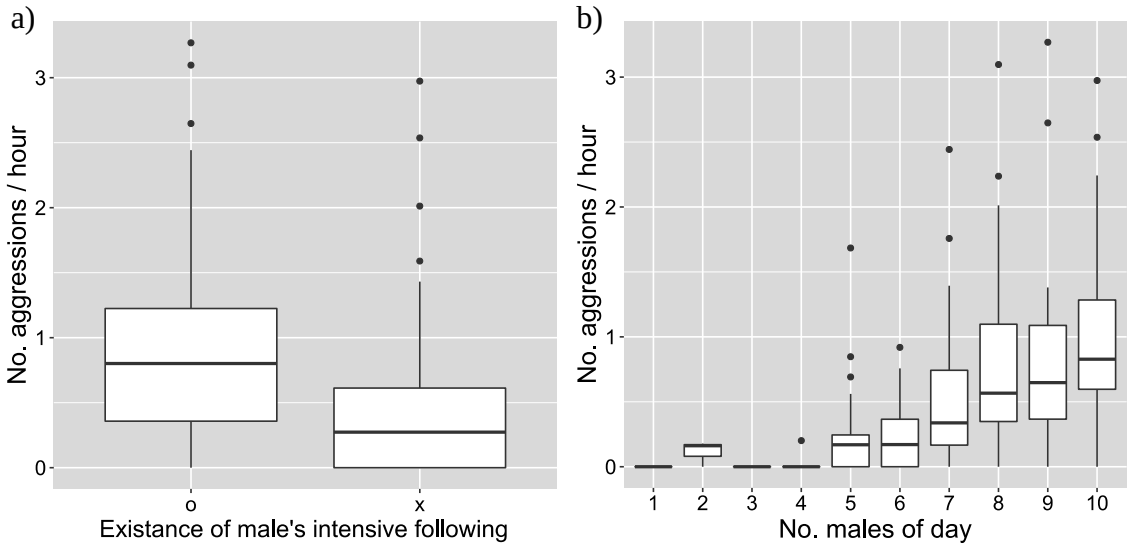
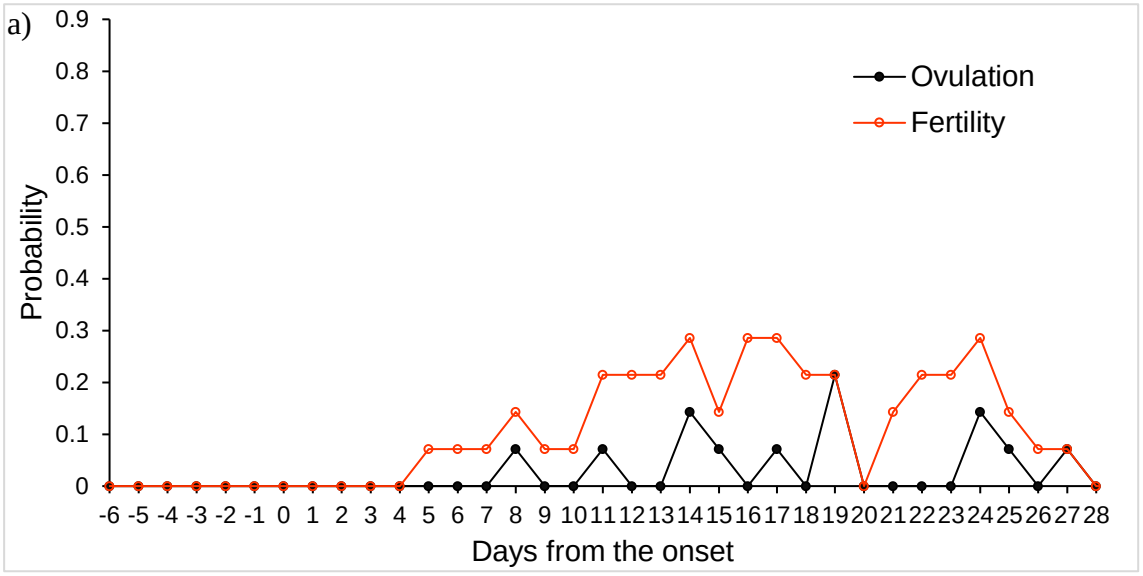


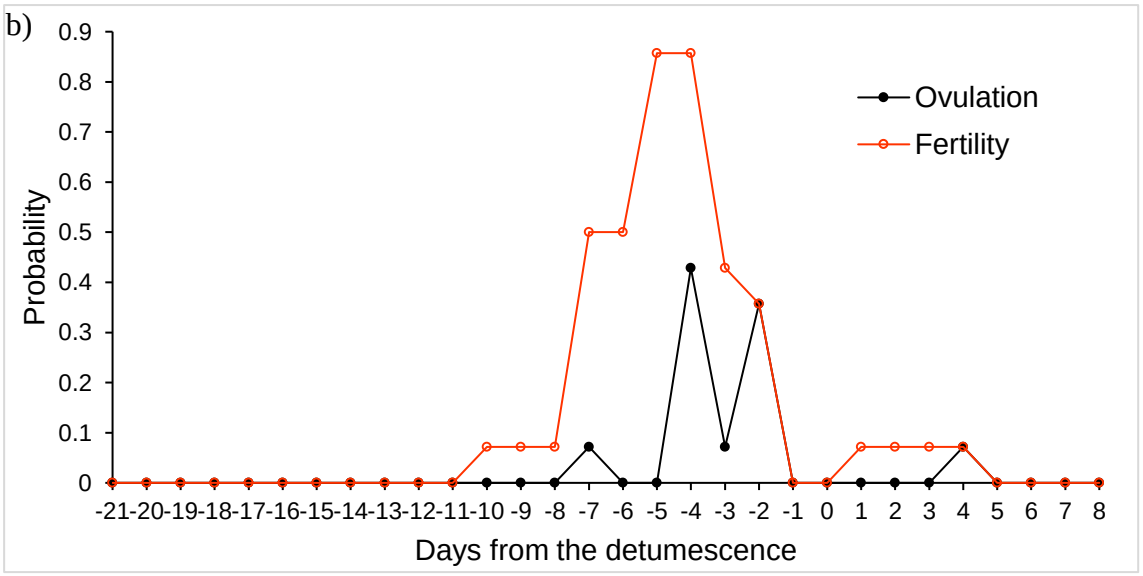
Figure 3-9. Probability of ovulation

The probability of ovulation in relation to days from the onset of maximal swelling phase (MSP) and days from the detumescence of MSP. It is important to note that the changes in fertility in figure 3-9a resembles the patterns in figure 3-4 which shows changes in the number of males in intensive following in relation to the onset of MSP.

a) Probability of ovulation and fertility calculated from the onset of MSP.

b) Probability of ovulation and fertility calculated from the detumescence of MSP.





4. Chapter 4

Prolonged maximal sexual swelling in wild bonobos facilitates affiliative interactions between females

4.1 Abstract

Perineal sexual skin swelling in relation to menstrual cycle occurs in a variety of primate taxa. However, sexual swelling with exaggerated size and color is found only in some Old World monkeys and the two *Pan* species. Although several hypotheses have been proposed (e.g., reliable indicator hypothesis and graded signal hypothesis), it seems unlikely that a single explanation can account for the significance of the sexual swelling in all of these species. Bonobos (*Pan paniscus*) provide an excellent opportunity for studying sexual swelling since they have the most prolonged maximal swelling periods among primates. In this study we propose a new hypothesis that sexual swelling in female bonobos increases their attractiveness to other females and thereby facilitates affiliative social interaction with them. We found that free-ranging female bonobos with maximal sexual swelling engaged in affiliative social interactions with other females, including genito-genital rubbing, staying in close proximity and grooming, more frequently than females without maximal swelling. These tendencies suggest that females with maximal swelling were attractive to other females. The results also suggest that the benefits of maximal swelling might vary among females depending on their life-history stage. In particular, young females may get more benefits from prolonged maximal swelling through increased grooming reciprocity and staying in close proximity to other females. Thus our study supported the hypothesis that one function of prolonged maximal swelling in bonobos is to increase attractiveness to other females, thereby enhancing affiliative relationships between females in a male-philopatric social system.

4.2 Introduction

The term sexual swelling is used to describe swelling of the skin of the perineum in relation to the menstrual cycle of females. It has been described in a variety of primate species including prosimians [e.g., ring-tailed lemur, *Lemur catta* (Jolly 1966), and Horsfield's tarsier, *Tarsius bancanus* (Wright et al. 1986)], Old World monkeys [e.g., Japanese macaques, *Macaca fuscata* (Tokuda 1961) and baboons, *Papio hamadryas* (Pocock 1906)], and ape species [e.g., bonobos, *Pan paniscus* (Savage-Rumbaugh and Wilkerson 1978; Kuroda 1980) and chimpanzees, *Pan troglodytes* (Zuckerman and Fulton 1934)].

Sexual swelling of very conspicuous size and color, which has been described as 'exaggerated sexual swelling' by Nunn (1999), is found only in several Old World monkey species and the two *Pan* species. In the rest of this paper, the term 'sexual swelling' is used synonymously with Nunn's (1999) definition of the exaggerated sexual swelling. It has also been suggested that this conspicuous sexual swelling has evolved independently in Old World anthropoids at least three times (Dixson 1983; Nunn 1999). In many species, sexual swelling reaches its maximal size near ovulation [e.g., *Macaca nigra* (Dixson 1977); *Papio anubis* and *Papio cynocephalus* (Hendrickx and Kraemer 1969); and *Pan troglodytes* (Dahl et al. 1991)] and most mating activity is concentrated in the maximal swelling periods (Saayman 1970; Furuichi 1987; Bercovitch 1988; Wallis 1992; Young et al. 2013). Sexual swelling is thought to incur some cost for females, such as possible hindrance of metabolism from water retention (Nunn 1999), increased chance of injuries (Hausfater 1975) and increased body weight (Bielert and Busse 1983), which can increase the energetic cost of locomotion (Nunn 1999). Therefore, it is assumed that there must also be advantages to females of having sexual swelling that outweigh the costs (Nunn 1999; Zinner et al. 2004; Alberts and Fitzpatrick 2012).

Several hypotheses have been proposed to explain the function and evolution of sexual swelling in the context of female reproductive strategies. Some researchers have suggested that sexual swelling invokes frequent promiscuous mating and inter-male competition that are beneficial for females [the best-male hypothesis (Clutton-Brock and Harvey 1976); many-male hypothesis (Hrdy and Whitten 1987)]. Others have suggested that the sexual swelling encourages dominant males to mate-guard and recognize paternity by accurately indicating the timing of ovulation, and thus to provide support for the females and/or offspring [obvious-ovulation hypothesis (Hamilton 1984); male-services hypothesis (reviewed in (Nunn 1999); and paternal care hypothesis (Alberts and Fitzpatrick 2012)]. Other explanations include a comprehensive hypothesis which puts together the functions of sexual swelling derived from several hypotheses, called graded signal hypothesis (Nunn 1999) and the reliable quality indicator hypothesis (Pagel 1994) which assumes that sexual swelling may indicate reproductive value of females. Furthermore, some groups of researchers have suggested variations of a social passport hypothesis. For example, that sexual swelling facilitates between-group transfer of adolescent females by showing resident males their oestrous status (Anderson and Bielert 1994), increases support from males in female–female competitive situations especially during immigration (Boesch and Boesch-Achermann 2000), or helps female–female interactions (Paoli et al. 2006). However, it seems unlikely that a single benefit inferred from a single hypothesis could account for all aspects of the phenomenon in all taxa. Although sexual swelling is found only in species with multi-male societies (Clutton-Brock and Harvey 1976), social systems vary among those species. It seems likely, therefore, that benefits of sexual swelling for females also differ between species and should be considered in the context of the social system in which they occur.

Bonobos (*Pan paniscus*) provide a unique opportunity for examining the function of sexual swelling as they have very prolonged periods of maximal swelling (Thompson-

Handler et al. 1984; Furuichi 1987; Dahl et al. 1991; Kano 1992) and also females that achieve high social status in a male philopatric society (Furuichi 1997; Surbeck et al. 2011; Furuichi 2011). In many primate species sexual swelling reaches its maximal size near ovulation (Wildt et al. 1977; Dahl et al. 1991; Emery and Whitten 2003; Young et al. 2013) and affects male behaviors related to mating, such as consortship (Tokuda 1961; Hall and de Vore 1965; Hill 1987) or coercive mate guarding (Smuts and Smuts 1993; Muller et al. 2007). However, bonobos seem to deviate from this general trend, since the timing of their ovulation and onset of maximal swelling are highly variable (Furuichi 1987; Heistermann et al. 1996; van Schaik et al. 2000; Reichert et al. 2002), but also see (Surbeck et al. 2011) even compared with those of chimpanzees (Deschner et al. 2003). The high level of variation of timing of ovulation and onset of maximal swelling in this species seem to reflect effects of very prolonged periods of maximal swelling (Furuichi 1987) of female bonobos. Therefore the key to understanding sexual swelling in bonobos may be found in the prolonged period of maximal swelling, which sometimes continues for more than 20 days (Furuichi 1987); Ryu, unpublished data).

Most previous research on the prolonged period of maximal swelling in bonobos has focused on its role in controlling the behavior of males, by making females attractive to males even outside the estrous periods (Furuichi 1987; Kano 1992; de Waal 1995; Furuichi 2011). This manipulative function of sexual swelling has been noted with the fact that there is no known case of coercive mate guarding and infanticide by males in bonobos (Furuichi and Hashimoto 2004; Paoli 2009). It is, therefore, recognized as a successful female counter-strategy toward male mating strategy. However, without considering that female–female and female–male bonding play an important social role and that sexual swelling seems to have an important role in social bonding (Parish 1994; Furuichi 2011) in this male-philopatric species, it will be impossible for us to reach a comprehensive understanding of the role and

evolution of sexual swelling. Therefore it is important to take, not only mating-related function, but also function for social bonding into account for better understanding. This will provide a new direction and another important dimension for future studies. Some researchers have already mentioned other possible functions of this prolonged sexual swelling, such as maximal sexual swelling for genito-genital rubbing (hereafter g-g rubbing; Kuroda 1980) rather than copulations (Takahata et al. 1996), or providing protection against male coercion by attracting other females rather than males (Paoli et al. 2006; Paoli 2009), but these have not been considered thoroughly, or examined quantitatively. Considering the finding that g-g rubbing increases for females with maximal swelling (Kano 1992; Hohmann and Fruth 2000), but also see (Takahata et al. 1996), it is reasonable to assume that sexual swelling influences female–female social relationships in bonobos (Kano 1992; Takahata et al. 1996; Paoli et al. 2006). In bonobos, new immigrant females, which are likely to have no stable social relationship with others in the group, are eager to interact with senior females (Furuichi 1989; Idani 1991), and such new immigrant females show very long-lasting maximal swelling (Furuichi 1987). Therefore, if females can gain benefits from having maximal swellings in terms of their social interactions with other females, those benefits are likely to vary in relation to their life-history stage.

Based on these characteristics, we hypothesized that one function of prolonged sexual swelling in bonobos is to increase attractiveness to other females and facilitate social interaction with them (**Swelling for female attraction**). This hypothesis leads to the following predictions:

1. Females with maximal swelling will be invited and/or engage in g-g rubbing more frequently than females without maximal swelling.
2. Females with maximal swelling will have more females in close proximity to them than females without maximal swelling.

3. Females with maximal swelling will spend more time in grooming interactions with other females than females without maximal swelling.
4. Young females with maximal swelling will receive more reciprocated grooming in interactions with other females.

To test this hypothesis and its predictions, we first investigated frequency of g-g rubbing as well as copulation in relation to age and swelling status. Second, we compared the number of females found within 1 m or 5 m of the focal animal, and those found in the same party. Finally, we investigated the frequency and reciprocity of grooming interactions in relation to age and swelling status of females.

4.3 Materials and Methods

Study site and subjects

Data were collected at the long-term bonobo field site at Wamba in the northern sector of the Luo Scientific Reserve, D.R. Congo (Kano 1992; Furuichi et al. 2012). The research camp is situated in the center of the northern section of the reserve (0°11’07.6’’N, 22°37’57.5’’E; WGS84). One fully habituated unit-group (or community) of free-ranging wild bonobos, called E1 group, was followed. From 1974 to 1996 researchers conducted field research using provisioning. Field research was stopped in 1996 by the civil war. Since researchers resumed field research in 2003, no artificial food has been provided. E1 group ranges over primary, old and young secondary, and swamp forests and sometimes agricultural fields (Mulavwa et al. 2010). January and February have less rain (Mulavwa et al. 2008), although there is no clear division of dry season and rainy season.

The E1 group consisted of 28 to 31 individuals, including 7 adult and 3 adolescent males and 9 adult and 2 adolescent females during the study periods. Nine adult females, whose estimated ages ranged from 14 to 41 years old in 2012, were the targets of focal sampling

(Altmann 1974). All of them had immigrated into the E1 group and had successfully given birth at least once after their immigration. Among the 9 adult females, 2 females who were more than 38 years old (Table 4-1) were classified as old, and 4 females from 21 to 29 years old were classified as middle-aged. Three females of less than 20 years old were classified as young. All of the young adult females had just one record of successful parturition in the E1 group after their immigration and had an infant during the study periods. One of 2 adolescent females (Nc) was the daughter of the oldest female (No) and the other (Zn) immigrated in October 2011, and they were not included in focal sampling. Hs and Sl were already members of the E1 group at the time that field study was resumed in 2003, therefore their immigration was earlier than August 2003. Yk and Jk joined the E1 group in 2004 with infants, which was considered a possible example of group fusion (for more details, see Hashimoto et al. 2008).

Assessment of sexual skin swelling

Sexual swelling was scored on a daily basis in relation to firmness and size of each individual (Furuichi 1987). Based on these records, the sexual swelling status of females was assigned to one of three categories: non-swelling (sw1), intermediate swelling (sw2) and maximal swelling (sw3). Firmness of sexual swelling was the key feature used to distinguish sw3 from sw2 since the size of swelling between 2 and 3, especially of young females, was not very different. Moreover because of great individual variation in swelling size, it has been suggested that firmness is a more reliable measure of sexual swelling in bonobo than size (Furuichi 1987). To avoid observation errors and individual bias in the assessment of sexual skin swelling, at least one researcher and two research assistants discussed the swelling status of each female during field observations and during daily meetings in the evening. Sexual swelling of one female (Sl) did not reach sw3 during the study periods. This might reflect its

pregnancy and postnatal infertility since it gave birth in December 2012 and was nursing its infant until the end of the second study period.

Behavioral observation

Data were collected by the first author from September 2011 to January 2012, and June to September 2012. During the study period, bonobos were followed for 1004.6 h on 134 days (7.57 ± 2.46 h per day). Bonobos were followed from their night beds, located before 06:00 h, until they made new night beds, usually later than 17:00 h, by one or two researchers and at least two research assistants (one mainly followed the main party and the other usually helped researchers). Bonobos live in fission-fusion social systems (Kuroda 1979; Kano 1982; White 1988), in which the composition of the membership of a party changes continuously (Aureli et al. 2008). When they split into several parties during daily ranging, the largest party was followed as far as possible. Party composition was recorded every single hour during party following (the 1-h party method; Hashimoto et al. 2001).

While bonobos were followed, one of the females in the party was randomly selected for continuous focal sampling. A random order for focal sampling for each day was generated before the beginning of daily observation. After bonobos were located in the morning at their bed site, I and one research assistant looked for the first animal in the random sampling order for at least 30 min. If we could not find the first one, we moved to the next one in the order, and so on. Some females had priority for focal animal selection because of a shortage of focal sampling data for them which was mainly due to the fission-fusion social system (sometimes some females were not observed for more than a week). One focal session was continued for 20 min and if a focal animal could not be located for more than 5 min within a focal session, the focal sampling was terminated. To keep a degree of independence of focal sampling of the same individual, focal follows for the same individual were separated by at least 100 min.

Within a focal session, all activities (e.g., feeding, moving, resting, grooming, agonistic interactions) involving the focal animal were recorded. All rare events, such as agonistic interactions (e.g., bite, hit, push and chase), copulations (Hashimoto 1997) and g-g rubbing (Kuroda 1980), which were observed during focal animals and parties following, were recorded. At the beginning of a focal session and every 5 min within the session (in total 5 scan points in a focal session), the names of neighboring individuals within 5 m of the focal animal and their behaviors were recorded. The total focal following time for all individuals was 241.99 h (744 focal sessions; 26.89 ± 0.39 h per individual) during 1004.3 h of party following on 134 days (7.57 ± 2.46 h). The average length of a focal session was 19.5 ± 1.1 min and each focal session consisted of 4.83 ± 0.42 scans (3591 scans in 744 focal sessions). More details for observed hours of each individual in relation to swelling status are shown in Table 4-1.

Data analysis

All analyses were done using R 2.15.2 (R Core Team 2012) in R-studio 0.97.248 (RStudio 2012) with the following packages: lme4 (Bates et al. 2012), glmmADMB (Sakug et al. 2012), nlme (Pinheiro et al. 2012), lmtest (Zeileis and Hothorn 2002), plyr (Wickham 2002), fitdistrplus (Delignette-Muller et al. 2012), ggplot2 (Wickham 2009). We used generalized linear mixed models (GLMM) or linear mixed models (LMM) to control for random effects of individual differences in the models. All models included swelling status (3 levels; non-swelling, intermediate swelling, maximal swelling) and age classes (3 levels; old, middle, young) as predictor variables. Interactions between swelling status and age classes were also examined in the models by visual inspection rather than by numerical inspection with data explorations, to avoid too many variables in the model.

Analysis of copulation and genito-genital rubbing (g-g rubbing)

To compare the frequency of copulation and g-g rubbing of females in relation to sexual swelling status, we used a generalized linear mixed model with negative binomial distribution (GLMM; Zuur et al. 2009). To examine the effects of swelling status and age on frequency of copulation and g-g rubbing (response variables), we included swelling status (3 levels: sw1–3) and age class (3 levels: young, middle, old) as predictor variables and ID of individuals as random variables in the model. Repeated copulations within the same dyad within 5 min (without any interruptions by others or copulation with others between repeated copulation) were considered to be one event. The number of copulations and g-g rubbing interactions on each day for each female were response variables. Most of copulations and g-g rubbings which occurred within an observed party were detected by researchers and research assistants. However, because of the fission-fusion social system, daily observed time for each individual was different from each other. To control for the difference of observation time among individuals, we included the number of 1-h parties in which each female was observed in a day as an offset term in the model.

Analysis of neighboring female pattern

To examine whether a female with maximal swelling attracted more females than females with other swelling status (non-swelling or intermediate swelling), the number of females in proximity to the focal females was analyzed using GLMM with negative binomial distribution. Two separate GLMMs were conducted, including the number of females within 1 m and 5 m respectively in response variables, with swelling status and age classes as predictor variables. Because one focal session consisted of several scan points (4.83 ± 0.42 scan points), each scan point could be related to others to some degree. To control for this spatio-temporal autocorrelation, each scan in a focal session was nested in a focal session and

the IDs of focal individuals were included as random variables in the model. Because it was also possible that spatio-temporal distribution of females in a 1-h party could have some effect on the neighboring females, the number of females in a 1-h party (response variable) in relation to swelling status and age class (predictor variable) were also examined by running another GLMM.

Analysis of grooming interactions

Because bonobos almost exclusively engaged in dyadic grooming interactions and very rarely in mutual grooming interactions (Sakamaki 2013), we analyzed only dyadic grooming interactions without mutual grooming (mutual grooming was observed only once in one dyad for 40 s in this study). To examine the effect of sexual swelling and age on the grooming interactions of females (which might reflect sociality of females), first, we examined occurrence of grooming in each focal session (response variable 0 or 1) in relation to swelling status and age class (predictor variables) by running a GLMM. Second, we compared the grooming reciprocity index (response variable) only in the focal sessions in which grooming interaction was observed using a linear mixed model (LMM), to examine the effect of sexual swelling and age class (predictor variables) on grooming reciprocity. In these two-step analyses, individual differences were controlled by including them as a random effect of the model. The grooming reciprocity index was calculated as follows:

$R(A) = \{Ge(B) - Gr(A)\} / \{Ge(B) + Gr(A)\}$

R(A): Grooming reciprocity index of A, while A was a focal animal (grooming with B)

Gr(A): $\sum$ (A groomed B, while A was a focal animal)

Ge(B): $\sum$ (A groomed by B, while A was a focal animal)

The value of the grooming reciprocity index: R(A) ranges from -1 (which means that A groomed B, but A was not groomed by B at all during the focal session) to 1 (which means that A was groomed by B, although A did not groom B at all).

4.4 Results

Copulation in relation to swelling status and age

In total 275 copulations were observed involving 9 adult females and 10 adult and adolescent males. These comprised 208 copulation events, when copulations that were repeated within 5 min were counted as one event. Females with maximal sexual swelling had more copulations than females with any other sexual swelling status (Figure 4-1a; for more details of GLMM, see Table 4-2). Young females also had more copulations than middle-aged or old females (Figure 4-1a; for more details of GLMM, see Table 4-2). The two old females did not copulate outside of their maximal swelling periods.

G-g rubbing and solicitation of g-g rubbing in relation to swelling status and age

184 cases of g-g rubbing were observed involving the 9 adult females. Females with maximal sexual swelling engaged in g-g rubbing more frequently than females with non-maximal swelling (Figure 4-1b; for more details of GLMM, see Table 4-2). There was no relationship between frequency of g-g rubbing and age. G-g rubbing typically in feeding contexts, such as immediately after entering a fruiting tree, or after encountering a food item (89.8%; 141 out of 157 g-g rubbing interactions in which context could be clearly confirmed). Because solicitation or initiation of g-g rubbing was confirmed in only 54 cases out of 184 (29.3%), we did not conduct statistical analysis for solicitation of the interaction. However, females with maximal swelling solicited other females with maximal swelling most frequently; in 25 of 54 g-g rubbings (44.64%). The second most frequently observed

solicitation was between females with intermediate sexual swelling (16.07%), and the third most frequently observed one was females with intermediate sexual swelling soliciting females with maximal swelling (14.29%). Solicitation between other combinations of females was less than 10% for each.

Females in the proximity and in the same party

Focal females had other females within 1 m (including females in contact with the focal females) in 17.3% of scans (621 of 3591), and the mean number of females within 1 m was 0.21 ± 0.51 (Figure 4-2a). Focal females had other females within 5 m in 40.8% of scans (1465 of 3591), and the mean number of females within 5 m was 0.63 ± 0.93 (Figure 4-2b). Focal females had 3.39 ± 2.23 female companions within the same 1-h party (Figure 4-2c). Females with maximal sexual swelling had more females within 1 m than those of other swelling status (GLMM; Table 4-3). Females with maximal swelling did not have females within 5 m compared with non-swelling females, although there was a significant difference compared with females with intermediate swelling (GLMM; Table 4-3). Females with maximal swelling, joined larger parties which consisted of more females than non-swelling females (Table 4-3), but there was no significant difference compared with females with intermediate swelling (Table 4-3).

Grooming frequency

Grooming interactions involving the focal animal were observed in 14.9% of focal sessions (111 focal sessions of 744). Females spent 8.69% of all focal animal following time (20.99 focal following hours of 241.99) in grooming interaction (sw1: 7.71%; 5.79 h, sw2: 6.93%; 7.17 h, sw3: 12.74%; 8.04 h, respectively). Comparison of the number of focal sessions (only in which grooming interaction was observed) in relation to swelling status

showed that females with maximal swelling engaged in grooming interactions more often than females of other swelling status [non-swelling: 12.6% (29 focal sessions of 230), intermediate swelling: 12.8% (41 focal sessions of 320), maximal swelling: 21.1% (41 focal sessions of 194); Figure 4-3a, GLMM; Table 4-4]. Young females engaged in grooming interactions more often than old females, but not more than middle-aged females (GLMM; Table 4-4).

Grooming reciprocity

The grooming reciprocity index was calculated independently for 111 focal sessions. Although old and middle-aged females seemed to have a higher overall reciprocity index (old: 0.275, middle: 0.078, young: −0.332) which might imply that they received more grooming than young females, this difference was not statistically significant (Figure 4-3b, LMM; Table 4-4). Females with maximal swelling had significant higher reciprocity index scores than those of intermediate swelling status (LMM; Table 4-4), but no difference was found compared with non-swelling status (LMM; Table 4-4). Although increase of reciprocity index of young females according to swelling status appeared to be different from the tendencies found in the other two age classes (Figure 4-3b), interaction between the two predictor variables (age class and swelling) was not statistically significant. However, further analysis with linear mixed model (LMM) on each age category revealed that there was no difference in grooming reciprocity index in middle-aged and old females in relation to sexual swelling, but there was a significant difference among swelling phases in young females. The pairwise test with LMM showed that the reciprocity index in young females was significantly higher in maximal swelling than in intermediate swelling ($p = 0.013$, $t = -2.599$, $SE = 0.184$), though the difference between maximal swelling and non-swelling was a non-significant tendency ($p = 0.097$, $t = -1.695$, $SE = 0.098$).

4.5 Discussion

Female bonobos are highly social (Furuichi 1987; White 1988; Kano 1992; Hohmann et al. 1999) and more central in their society than female chimpanzees (Furuichi 1989; Kano 1992; Parish 1994; Surbeck et al. 2011; Furuichi 2011). Previous studies have suggested that prolonged sexual swelling of this species may contribute to the more female-centered society of bonobos by diminishing the competitive element of male mating strategy (Surbeck et al. 2011; Furuichi 2011). Although this study also supported the idea that sexual swelling attracts males as a sexual signal, the four predictions concerning female-female interactions were also supported. Thus our results support the hypothesis that the prolonged sexual swellings of female bonobos also facilitate female-female interactions by making females with maximal swelling more attractive to other females. Therefore, this study suggests that prolonged sexual swelling might be a physiological adaptation in this species to maintain high level of affiliative social bonding among females and may contribute to high social status of females in the society (de Waal 1995; Parish et al. 2000; Furuichi 2011). In addition, the function of sexual swelling in bonobos and chimpanzees may differ in that sexual swelling in chimpanzees seems to function mainly to attract males (Boesch and Boesch-Achermann 2000; Hashimoto et al. 2001). It would be very useful to examine the extent of variation between populations or sub-species in chimpanzees and bonobos for a better understanding of the function of sexual swelling in *Pan* species.

Increased socio-sexual behavior during the maximal swelling period of females

Our data showed that the frequency of copulation was clearly related to the swelling status in all age classes, which is consistent with previous reports from wild populations (Furuichi 1987; Surbeck et al. 2011). Although some captive studies have reported that females copulated with males throughout swelling cycles, with the exception of a few days

(Savage-Rumbaugh and Wilkerson 1978; de Waal 1995), this study showed that this tendency was prominent for young females only. Young females (less than 5 years of tenure) had higher copulation frequencies than other females even in non-maximal swelling periods. By contrast, two old females never copulated with males outside of maximal swelling status. This result may indicate that the need for socio-sexual activity is greater for young females than old females, because they have relatively short tenure in the group and therefore, may be more vulnerable to competition even with males. On the other hand, it could be that they need male support in agonistic interactions with other females, which is predicted by the social passport hypothesis (Boesch and Boesch-Achermann 2000; Deschner and Boesch 2007).

Considering the fact that g-g rubbing was most frequent in female dyads with maximal swelling and this interaction occurs most frequently in feeding contexts (89.8% in this study, 79.5% in Hohmann and Fruth 2000), females with maximal swelling can take advantage of their condition in feeding contexts. In particular, young females, whose rank is usually low (Furuichi 1997; Ryu, unpublished data) and are, therefore, at a disadvantage in feeding competition, can gain more benefits from keeping maximal swelling since genito-genital rubbing requires body contact and therefore decreases distance and may also reduce tension between two participants (Furuichi 1989; Hohmann and Fruth 2000). This may also explain the benefits and the function of very long-lasting maximal swelling of new immigrant females (Furuichi 1987; Furuichi 1992) although they were not the subjects of this study.

Increased social interaction and attractiveness of females with maximal swelling

Females with maximal swelling tended to have more females in proximity, and this tendency was more significant in 1 m proximity, which corresponds to the ‘personal distance’ defined in the proxemics as established by (Hall 1966). Females with maximal swelling also had more females in the same 1-h party, although the difference between intermediate

swelling and maximal swelling was not significant. These results suggest that females with maximal swelling attended bigger parties, or that they attracted other females to attend parties, although it was not possible to distinguish between these two possibilities in this study.

It has been reported that time spent in proximity is highly correlated with frequency of affiliative social interaction in primates [rhesus macaques (*Macaca mulatta*) (Hill 1987); baboon (*Papio cynocephalus*) (Silk et al. 2003); ring-tailed lemurs (*Lemur catta*) (Sbeglia et al. 2010)] and, thus, staying in very close proximity, such as within 1m, probably facilitates social interaction or at least indicates mutual or one-directional attraction among females. Therefore, together with grooming interaction and g-g rubbing data including solicitation of interaction, the increased number of females within 1 m of females with maximal swelling implies that females with maximal swelling were attractive for other females for social interaction.

This study also suggested that sexual swelling played a positive role in grooming interactions. The high frequency of grooming interactions of young females is also compatible with previous reports that young females had higher needs of social bonding with other females than older females (Furuichi 1989; Idani 1991). This implies that the benefits and reason to keep maximal swelling might vary in relation to female age. Although young females showed lower grooming reciprocity indices in general, it shifted to almost the same as old and middle-aged females when they had maximal swelling.

Newly-immigrated young female bonobos show very prolonged periods of maximal swelling (Furuichi 1987; Furuichi 1992) which were longer than adult females which are already settled in the group. In chimpanzees, it has been suggested that maximal swelling of new immigrant females is to attract males to facilitate the immigration process (Boesch and Boesch-Achermann 2000). However this study provides an additional scenario in which

maximal swelling of new immigrant females in bonobos is also used to facilitate female–female social interaction as well as male attraction. Increasing the periods of maximal swelling may help new immigrant females to integrate into the new group more easily. If females with maximal swelling are more socially active and attract other females for social interactions with them, prolonged periods of maximal swelling are more beneficial for immigrant young females whose needs of social bonding with more senior females seem to be greater (Furuichi 1989; Idani 1991).

Grooming in primates has been studied intensively and has been shown to have important social functions in maintaining and strengthening social relationships (Seyfarth 1977; McKenna 1978; Hill 1987; Spruijt et al. 1992), regulating stress and tension (Spruijt et al. 1992), and may also function as currency exchanged for tolerance or agonistic support (Henzi and Barrett 1999). Therefore, increased reciprocity in grooming interactions of young females with maximal swelling may result in direct benefits such as reducing stress, ecto-parasite load and facilitating social bonding. It may also help young females to strengthen social bonding with other females in the long-term. Such benefit seems to be greater for bonobos, in which senior females tend to have high social status and play central social roles (Parish 1994; Parish 1996; Furuichi 2011).

Overall, this study suggests that one of the keys to understanding the function of sexual swelling in bonobos (especially for young females) is increased attractiveness of females for social interactions during prolonged maximal swelling periods. Female bonobos show prolonged periods of maximal swelling throughout their lifetimes (ca. 27% of time inter-birth interval within the average 4.8 years birth interval; Furuichi and Hashimoto 2002). However, it is not reasonable to assume that these prolonged periods of maximal swelling evolved only for male attraction, since female bonobos showed lower copulation rates than female chimpanzees (Furuichi and Hashimoto 2002) during maximal swelling periods and also

females can be receptive even in non-maximal swelling cycles, as was found in young females in this study.

Therefore we suggest, in conclusion, that attraction of other females has become an additional function of sexual swelling in bonobos, which originally evolved as a sexual signal to males. In other words, prolonged periods of maximal swelling in bonobos may have evolved partly to facilitate social interaction among females and may contribute to a female-centered social structure by facilitating female–female bonding in a male-philopatric society (White 1988; Parish 1994; Surbeck et al. 2011; Furuichi 2011). Together with the results of this study and previous suggestions that sexual swellings have disappeared and re-emerged at least three times during speciation (Nunn 1999), the function of sexual swelling may differ among species. Therefore, we suggest that it is useful to consider the demography and socio-ecological factors of each species for a more comprehensive understanding of the function and evolution of sexual swelling in Old World monkeys and apes.

During the long life-span of bonobos, they encounter demographical and socio-environmental changes which have to be dealt with in order to live in the group and minimize fitness loss. Therefore it seems to be more advantageous for females if they have some degree of flexibility in the length of maximal swelling periods in relation to their life-history or socio-environmental or demographical changes since sexual swelling also incurs some costs. Although it has yet to be investigated thoroughly and quantitatively, there seems to be some degree of flexibility and individual difference in occurrence of maximal swelling (Furuichi 1987; Furuichi 1992). Long-term field study will clarify this speculation about the flexibility of keeping maximal swelling and will provide valuable perspectives on understanding female sexuality and its evolution, not only in this species, but also in humans.

4.6 Authors' contributions

Conceptualization, H. Ryu; Investigation, H. Ryu; Methodology, H. Ryu; Formal Analysis, H. Ryu; Writing – Original Draft, H. Ryu; Writing – Review & Editing, H. Ryu, D.A. Hill, T. Furuichi; Project Administration, T. Furuichi; Supervision, T. Furuichi, D.A. Hill; Funding Acquisition, T. Furuichi, H. Ryu (following Brand et al. 2015).

Table 4-1. Composition of females of E1 group and focal following hours (2012)

Name	Age	Age class	Immigration	Latest delivery	Focal following hour (party)		
					Swelling 1	Swelling 2	Swelling 3
No	41	Old	1983.11	2009.05	11.5 (218)	6.4 (148)	9.6 (307)
Ki	38	Old	1984.12	2009.07	4.3 (94)	15.3 (298)	7.8 (290)
Hs	29	Middle	> 2003.08	2009.08	6.9 (105)	12.9 (232)	7.1 (203)
Yk	29	Middle	2004.04	2009.10	7.1 (109)	10.2 (229)	9.8 (279)
Jk	24	Middle	2004.04	2012.01	16.7 (285)	7.4 (262)	2.9 (84)
Sl	21	Middle	> 2003.08	2011.12	9.5 (211)	17.1 (365)	-
Nv	17	Young	2007.08	2008.09	1.6 (47)	9.9 (199)	15.0 (353)
Ot	15	Young	2008.06	2011.02	1.7 (37)	17.5 (432)	7.3 (152)
Fk	14	Young	2008.06	2011.02	15.8 (326)	7.1 (264)	3.5 (121)

Age of each female was estimated based on immigration date and several morphological cues. All of the latest deliveries survived so all adult females had infants during the study periods. Each number in the following hour column refers total focal sampling hours for each individual in relation to swelling status (non-swelling: sw1, intermediate swelling: sw2, maximal swelling: sw3). Numbers in the parentheses show the total numbers of one-hour party segment in which each female was observed.

Table 4-2. GLMM results of copulations and genito-genital rubbing

Negative binomial GLMM results of copulation and genito-genital rubbing in relation to swelling status and age.

Predictor variables	Copulation				G-G rubbing			
	estimate	se	z	p (>|z|)	estimate	se	z	p (>|z|)
Intercept	-5.380	0.416	-12.94	< 0.001	-5.782	0.540	-10.71	< 0.001
Swelling 1 vs 3	-4.311	0.536	-8.04	<0.001*	-1.629	0.387	-4.21	< 0.001*
Swelling 2 vs 3	-2.357	0.383	-6.15	<0.001*	-1.403	0.334	-4.20	<0.001*
Middle-aged vs Young	-1.159	0.492	-2.35	0.019*	-0.185	0.677	-0.27	0.79
Old vs Young	-3.259	0.625	-5.21	<0.001*	-0.830	0.807	-1.03	0.30

Table 4-3. GLMM results of number of females in proximity

Negative binomial GLMM results of number of females within 1m, within 5m and within one-hour party in relation to swelling status and age.

	GLMM	Intercept	Swelling 1 vs. 3	Swelling 2 vs. 3	Middle-aged vs. Young	Old vs. Young
Within 1m	estimate	-2.517	-0.613	-0.788	-0.069	-0.349
	se	0.24	0.257	0.239	0.224	0.267
	z	-10.48	-2.39	-3.3	-0.31	-1.31
	p (>|z|)	< 0.001	0.017*	0.001*	0.756	0.192
Within 5m	estimate	-0.933	-0.174	-0.307	-0.03	-0.196
	se	0.18	0.166	0.142	0.205	0.244
	z	-5.18	-1.05	-2.16	-0.15	-0.8
	p (>|z|)	< 0.001	0.295	0.031*	0.883	0.422
Within party	estimate	1.471	-0.35	-0.091	-0.082	-0.038
	se	0.046	0.053	0.049	0.046	0.054
	z	31.93	-6.56	-1.86	1.79	0.71
	p (>|z|)	< 0.001	< 0.001*	0.063	0.073	0.473

Table 4-4. GLMM results of grooming reciprocity

Binomial GLMM results of comparison of the number of focal sessions which included grooming interactions and linear mixed model (LMM) result of comparison of grooming reciprocity index in relation to swelling and age.

Predictor variables	Focal session with grooming				Grooming reciprocity index			
	estimate	se	z	p (>|z|)	estimate	se	t	p (>|z|)
Intercept	-1.076	0.211	-5.10	< 0.001	-0.205	0.174	-1.182	0.240
Swelling 1 vs 3	-0.610	0.271	-2.26	0.024*	-0.072	0.147	-0.487	0.628
Swelling 2 vs 3	-0.612	0.246	-2.49	0.013*	-0.259	0.124	-2.077	0.040*
Middle-aged vs Young	-0.242	0.232	-1.04	0.296	0.340	0.221	1.541	0.174
Old vs Young	-0.668	0.300	-2.23	0.026*	0.630	0.266	2.372	0.055

Figure 4-1. Copulation and g-g rubbings per hour

Mean number of copulations and g-g rubbing interactions per hour with standard error.

(a) Copulations in relation to swelling status and age.

(b) G-g rubbing interactions in relation to swelling status and age.

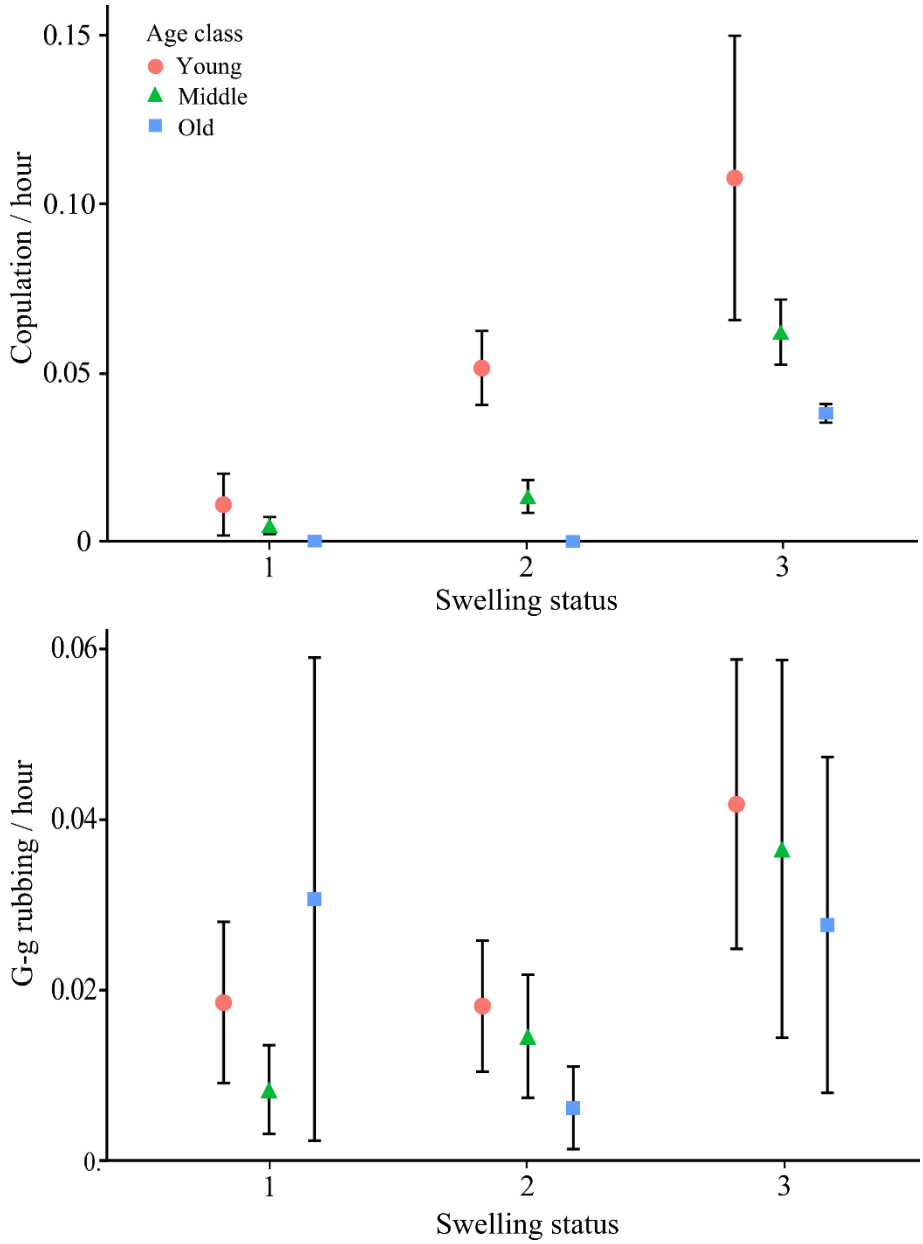


Figure 4-2. Mean number of females within 1m 5m and party

(a) Number of females within 1m in relation to swelling status and age.

(b) Number of females within 5m in relation to swelling status and age.

(c) Number of females in one-hour party in relation to swelling status and age.

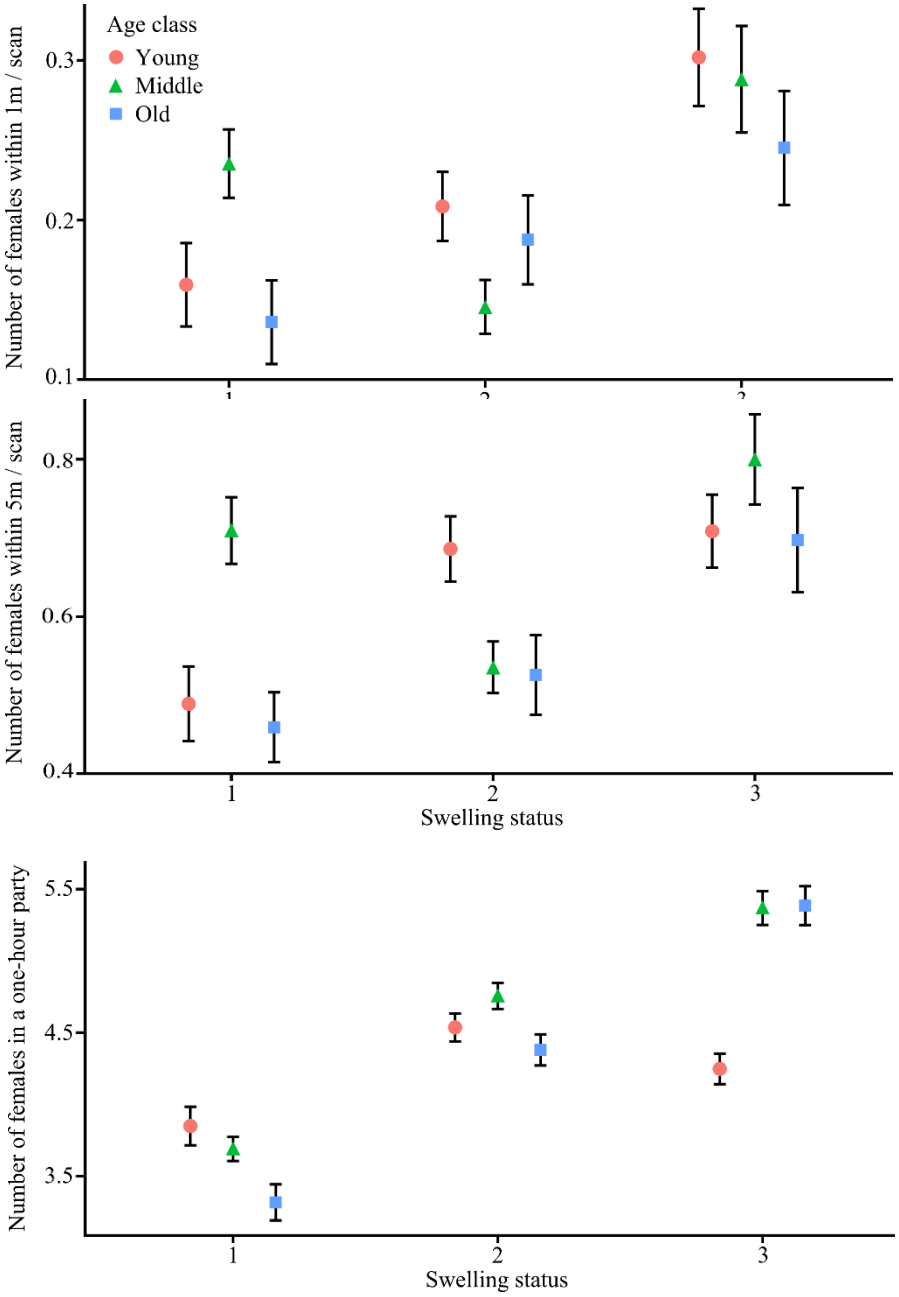
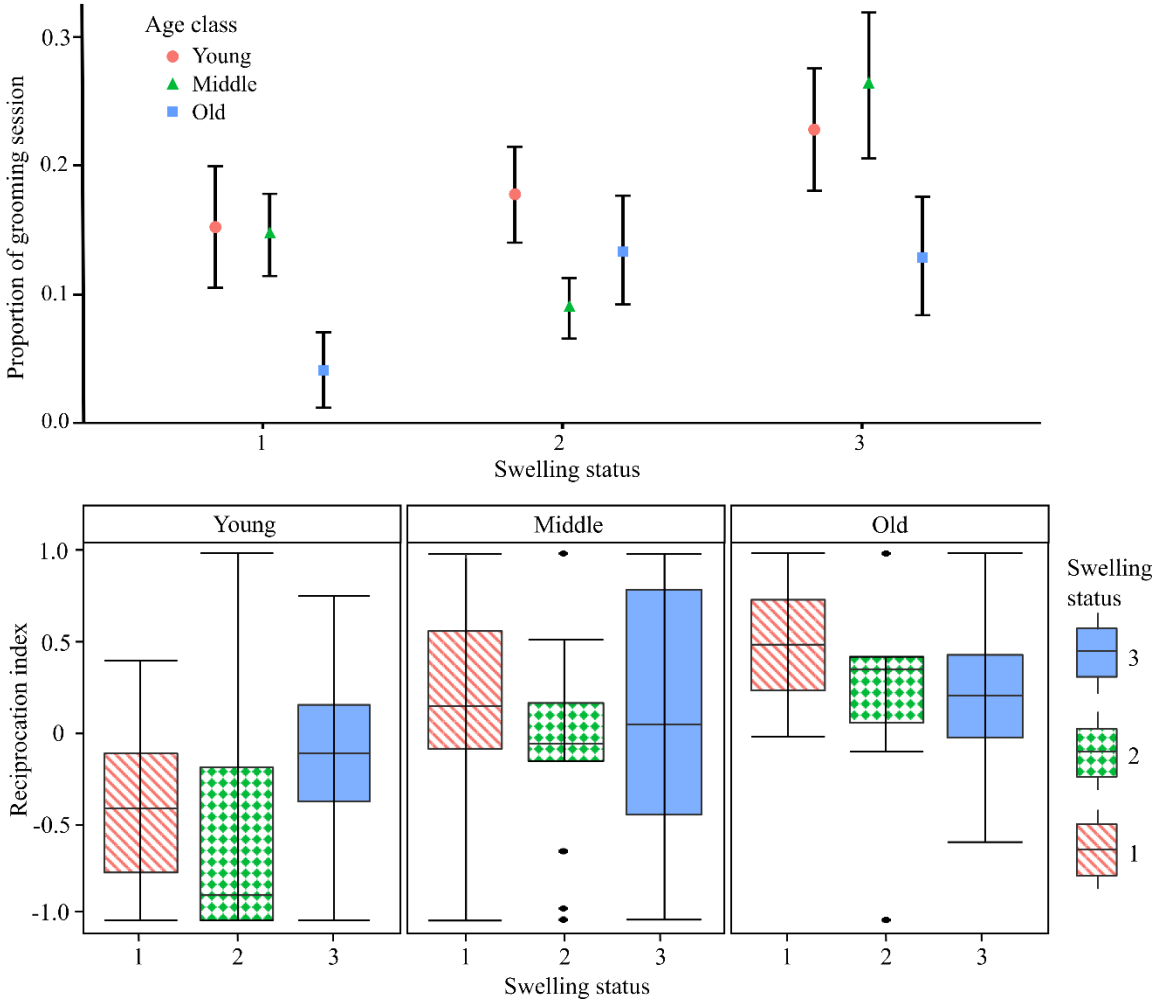


Figure 4-3. Grooming frequency and reciprocity

(a) Proportion of focal sessions in which grooming interaction was observed.

(b) Grooming reciprocity index. Each box indicates that upper quartile and lower quartile and dots indicate outliers.



5. Chapter 5

General Discussion

5.1 Summary of the result

In Chapter 2, as found in previous reports (Heistermann et al. 1996; Reichert et al. 2002), increases in estrogen coincided with maximal sexual swelling. The duration of the maximal swelling phase showed great inter- and intra-individual variation. Long-term data confirmed that female bonobos resume maximal swelling within 1 year of successfully giving birth (240.5 days). However, after a female's infant reached 2 years of age, it seemed that this variation stabilized (Figure 2-4). The proportion time during which females were in the maximal swelling phase increased with infant age. This might be because females with weaned infants can maintain their menstrual cycles until they get pregnant, or cycle more frequently than females with younger infants. Females with younger infants might experience temporal stoppages in their menstrual cycles more often, which has been reported in chimpanzees (Deschner and Boesch 2007). The length of the luteal phase, from ovulation to the first day of menstruation, was only 10.6 ± 0.6 days (3 cases) and resembled previous findings from another wild bonobo population (9.5 ± 1.2 days; Douglas et al. 2016).

In Chapter 3, I found that male bonobos allocate their mating efforts, including intensive following, solicitation and intervention of copulations, mainly to the females with high reproductive quality (i.e. to those with older infants and a high probability of ovulation). The number of copulations also increased with ovulation probability. However, infant age had a negative influence on the number of copulations. Using a reduced data set (Table 3-5), I showed that low ranking males copulated more with females that had younger infants regardless of the probability of ovulation. In contrast, the number of copulations of high ranking males increased with the probability of ovulation, though the infant age did not predict the number of copulations observed.

In the Chapter 4, I tested whether the sexual swellings of females have an additional function that facilitates female– female affiliative interactions. I found that genito-genital rubbing, which is a female–female affiliative interaction unique to bonobos, was more frequent between females with maximal swellings. The number of females within 1m from a focal female also increased when the focal female was maximally swollen. However, the number of females within the party of bonobos in which the focal sampling was being conducted also increased when the focal female exhibited maximal swelling. Therefore, spatio-temporal relationships between females might also cause this pattern. I also found that young females received more grooming from other females when they had maximal swellings.

5.2 Sexual swelling and ovulation

In this study, I reconfirmed that the role of estrogen and progesterone in the cyclic changes in the size of sexual swellings in bonobos resembles that of chimpanzees and other primates. My results also suggest that the considerable inter- and intra-individual variation in the length of the maximal swelling phase might in part be due to the lactation of females with young infants and early-pregnancy termination events (i.e. miscarriages) of females with older infants. The physiological condition of females influenced by these reproduction related events (lactation and early loss) might explain the poor predictability of ovulation from the onset of sexual swelling in bonobos (Reichert et al. 2002; Douglas et al. 2016; Street et al. 2016).

Indeed, the previous study (Douglas et al. 2016), which reported poor predictability of ovulation from the onset of maximal swelling in wild bonobos, included females with young infants (less than 2 years) and did not consider the possibility of early loss. The only other study to address this topic focused on captive bonobos and showed tighter connections

between sexual swelling and ovulation (16 of 23 cases; 69.6% of ovulations were took place in the second half of maximal swelling phase in Reichert et al. 2002). It is possible that the study by Reichert et al. (2002) included more females with older infants, resulting in higher predictability of ovulation than the study by Douglas et al. (2016). However, Reichert et al. (2002) did not provide detailed information of infant age, so I can only speculate.

According to current knowledge, including the findings in this dissertation, the reason that the maximal swelling is a poor predictor of ovulation might relate to the high inter- and intra-individual variation in the length of the maximal swelling phase. Though it is still possible that poor predictability is due to the difference in measurement in sexual swelling at different field sites, considering greater inter-intra variations in the length of maximal swelling phase in bonobos, this might not be the case. The high variation both between and within females in maximal swelling length can be explained by their respective reproductive histories, including lactation and early-loss (early-miscarriage), which influences body condition and thereby the follicular phase of females. Figure 5-1 is a conceptual diagram, which represents the occurrences of maximal swelling from a delivery to the next delivery.

Another factor that might underlie differences in cycle lengths between bonobos and chimpanzees relates to nutritional conditions. It has been suggested that bonobo habitats might be more productive than chimpanzee habitats (White and Wrangham 1988; but also see Furuich et al. 2015). Relaxation of nutritional constraints in bonobo habitat may therefore allow female bonobos maintain longer cycles and cycle more often. Habitat quality in terms of nutrient supply might also explain why female chimpanzees at Taï exhibit roughly twice as many swelling cycles as female chimpanzees at Gombe and Mahale (Deschner and Boesch 2007). The finding that fruit availability is positively related with the number of females with maximal swelling in chimpanzees (Anderson et al. 2006) further supports the hypothesis that nutritional conditions influence cycle length and frequency. The early resumption of maximal

swelling in female bonobos, which contributes to inter- and intra-individual variation in the length of maximal swelling phase, may thus be in part due to their nutrient-rich habitats.

Alternatively, sexual swellings of female bonobos might be more sensitive to estrogen than those of female chimpanzees. If this is the case, small increases of the hormone might result in maximal swelling in bonobos and might explain the early resumption of the maximal swelling phase in bonobos (Chapter 2). Apart from estrogen sensitivity, it seems that the morphology of sexual swellings in bonobos might also differ from that of chimpanzees (Figure 5-2). In chimpanzees, swellings are only just visible at the minimal swelling size. However, in bonobos swellings are readily visible even when the sexual skin swelling is at its minimum size. This might suggest that the function of sexual swellings in bonobos is somehow different from that of chimpanzees.

I compared the periods of maximal swelling of chimpanzees and bonobos with a linear mixed effect model using published data set, which was presented in Chapter 1 (Table 1-1). The mixed effect model included the study sites as a random effect, to control the discrepancy between the periods of maximal swelling phase from the same populations. The model showed that the period of maximal sexual swelling of bonobos (13.47 ± 1.98 days) is longer than compared with that (11.27 ± 1.14 days) of chimpanzees (Table 5-1 and Figure 5-3). However, since different studies used different methods for size measurement of the sexual swelling, this result should be interpreted with caution.

The prolonged and large variation in the duration of the maximal swelling phase in female bonobos (Chapter 1 and 2) might play a major role in confusing paternity. However, as discussed in Chapter 4, the sexual swellings of bonobos might also play an important role in facilitating female–female affiliative relationships.

In my study, evidence of ovulation (i.e. when PdG increased above the threshold and remained there for more than 3 weeks) was found in a female (Nv) when her infant was just

1.7 years of age (20 months). This was the earliest ovulation among the females with infants. However, due to a 5-day sampling gap, I could not use these data in the analysis, since the missing data caused by the gap made it impossible to specify the ovulation date and fertile phase. Five anovulatory maximal swelling cycles were also observed in females with young infants ranging in age from 12 to 35 months. This suggests that even though the sexual swellings of females with young infants (less than 2 years) reaches its maximal size, the maximal swelling phase does not necessarily coincide with ovulation.

In general, there was a tendency for the duration of the maximal swelling phase to increase with infant age (GLMM 1-b in Table 2-1). However, considering the residual heteroscedasticity of this regression, this linear relationship might be dominated by the numerous data points of very short maximal swelling from females with infants less than 1.5 years of age. There were also very long periods of maximal swelling from the females with young infants. This might also reflect the unstable and prolonged follicular phase, which also resembles results of previous studies (Douglas et al. 2016).

One concern of the present study is the inter-plate coefficient of variation (CV) for PdG, which was 15.83 (inter-plate CV for E1C was 10.79). However, this CV is very close to the acceptable level of 15% (p. 723 in (Auletta et al. 1978)). This CV probably does not cause a serious problem in determining the ovulation days, as I put samples from the same individual in the same plate as far as was possible, and only the average value of PdG was used to calculate the overall hormonal profile for the Figure 2-1.

5.3 Male response to females with different reproductive values

As discussed in Chapter 3, the poor predictability of ovulation might not be a big challenge for male bonobos. In this study, I demonstrated that male bonobos, like male chimpanzees (Deschner et al. 2004; Thompson and Wrangham 2008), concentrate their

mating efforts to the females with higher reproductive quality based on ovulation probability and infant age. If these findings concerning male behavior are the products of deceptive signaling of ovulation (also referred to as concealed ovulation; Turke 1984; Wrangham 1993; Reichert et al. 2002), manifest as prolonged periods of maximal swelling in female bonobos, it will be very interesting to determine how this unexpected response of males has evolved. It is also of interest to answer the question of how the highest ranking male sired more than 50% of the offspring in the study group(Ishizuka 2017) if males can be deceived by dishonest signals. Therefore, it is unlikely that males are deceived by the poor predictability of ovulation from the onset of maximal swelling. Instead, males might use a probabilistic approach and target females with the oldest infants as well as those whose maximal swelling phase started earlier (earlier onset) or are closer to detumescence. The three-layered model (Chapter 3) does not necessarily assume a male's ability to discriminate subtle changes in size within the maximal swelling phase if the signal is presented continuously. However, if males can discriminate subtle changes in size, this would provide added benefits toward their reproductive success. There may also be other cues by which males might predict ovulation, such as through various odors or behaviors of females, or even behaviors of other males if there variation in the ability of different males to detect ovulation. I hope these possibilities will be addressed in future research.

One contribution of the present study is that male–male competition over cycling females in bonobo does exist: male bonobos invest time and effort into outcompeting other males in relation to the changes in sexual swelling size of females. More specifically, males compete for mating opportunities and interfere with the mating opportunities of other males that involve females with high reproductive quality (with older infants and higher probability of ovulation). Sexual swellings, therefore, function as determinative signals for male bonobos in allocating mating efforts to periods with a high probability of ovulation. With this finding, it

is not easy to imagine that the poor predictability of ovulation caused by long maximal swelling phase has resulted in lower male-mating competition in bonobos, and therefore, the peaceful nature of bonobos. It is still possible that a higher number of females with maximal swelling in the group allows low-ranking males to mate with females with lower probability of conception, and this might regulate social tension between males. However, the number of copulations by low ranking males was small (Table 3-1b). This study could not examine the possibility of the socio-regulatory function of sexuality, which may be influenced by prolonged receptivity of bonobo females throughout the early resumption of maximal swelling during early lactation periods (Chapter 2).

Reproductive quality of females can be represented at broader time scales by the age of their infants, since the probability of conception will increase with infant growth. Also, the reproductive quality of females can be represented at a narrower time scale by the probability of ovulation within a menstrual cycle. Considering the fact that the average inter-birth interval of bonobos in the study group was 4.8 ± 0.9 years, it is reasonable for males to concentrate their mating efforts on females with older infant (probably over 3 years of age). By concentrating their mating efforts within the maximal swelling phase and allocating greater effort (mate guarding and increasing the number of copulations) approaching detumescence, high-ranking males can better ensure paternity than low-ranking males. The low-ranking males might have difficulty accessing fertile females in the group. However, in Chapter 3, I showed that even low-ranking males actively sought opportunities to copulate by intensively following females with high probability of ovulation and older infant, just as high-ranking males did. The behavior of low-ranking males toward females suggests that low-ranking males are mainly restricted to mating with females that have younger infants and whose ovulation remains distant. However, considering that human sperm – among the closest relatives of bonobos – can remain viable for over 3 days in the reproductive tracts of

females (Wilcox et al. 1995), the possibility of siring offspring is not zero even for low-ranking males if they can copulate with females in the fertile phase.

It is unlikely that females lack counter-strategies to male mating strategies, in particular those of high-ranking males, as the high-ranking males might want to monopolize the copulation of females, which then might influence female mate choice. Given that there is no evidence of coercive mating in male bonobos (Paoli 2009), females can be more selective and refuse mating with certain males. This might be more evident in aged females whose social rank is usually high (Furuichi 1997). However, such selectivity might also represent some cost for females, such as losing the chance to get pregnant earlier. Moreover, if sperm competition (Harcourt et al. 1981; Parker 1970) influences bonobo reproduction, female mate choice, which limits sperm competition, might result in lower reproductive success of their male offspring over generations.

5.4 An additional function of sexual swelling in bonobos

Why do female bonobos resume swelling within 1 year of giving birth and more frequently exhibit maximal tumescence (prolonged; see Chapter 1)? There might be several possible explanations for this earlier resumption of swelling as described in the previous section of this chapter. Energy constraints are likely to have a regulative role in the sexual swelling cycle (Thompson et al. 2012). This hypothesis may be supported by the finding that fruit availability is positively correlated with the number of females with maximal swelling in wild chimpanzees (Anderson et al. 2006). Also, chimpanzees in captivity, i.e. with fewer nutritional constraints, have a shorter period of post-partum amenorrhea than those in the wild (Courtenay 1987). Therefore, the early resumption of the maximal swelling phase in female bonobos seems to be related at least in part with lower energetic constraints coincident with higher habitat quality.

Also related noticeably if subtly to inter-individual differences between females in the resumption of sexual swellings following birth is their own age. It is possible that young and unexperienced female bonobos resume their maximal swellings earlier than older females, as has also been found in chimpanzees (Deschner and Boesch 2007), although our results in Chapter 2 did not reach statistical significance. If that is the case, this may be related to nursing inexperience (e.g. not nursing with sufficient frequency or duration) or the faster body recovery in younger females in comparison with older females. It is also possible that young females usually exhibit smaller sexual swellings, which might more easily reach its maximal size.

The last possible explanation for the early resumption of sexual swelling in bonobos is that this phenomenon was selected because of its social benefits to females. As demonstrated in Chapter 4, it seems that maximal swellings increase the attractiveness of females to both males and females. The social attention from other bonobos seems to play a positive role in that females with maximal swellings, particularly young females whose rank is usually low, receive more grooming and engage in more affiliative interactions such as genito-genital rubbing, in addition to generally having more individuals in close proximity.

If female bonobos can gain more benefits by exhibiting maximal swelling, it will be important to ask whether the periods of maximal swelling phase in bonobos have actually been selected to be prolonged? As presented in the previous section 5.2, the length of the maximal swelling phase might be somewhat longer and more variable in bonobos than in chimpanzees (13.47 days in bonobos and 11.2 days in chimpanzees).

Is this a meaningful difference? It was suggested that the prolonged periods are seemingly meaningless when the average sexual swelling cycle length of female bonobos is longer than that of female chimpanzees (Furuichi and Hashimoto 2002). With the present study, I cannot draw a concrete conclusion to this question. However, throughout frequent

exhibition of maximal swelling and occasional but very long maximal swelling phase, female bonobos can receive social benefits as well as exert greater influence on male mating behavior. It is not yet clear how a signal that evolved to attract males has acquired the function to attract other females. The key, however, might be related to the fact that genitalia are critical to reproduction and are highly sensitive. By using the genitalia in genito-genital rubbing, female bonobos may ensure that their relationships are stable and strong. The importance of the genitalia in this social behavior might then lead females to be interested in the sexual swellings of others. However, longitudinal data are needed to evaluate the possible mechanism(s) driving female attraction to sexual swellings. In humans, women are also attracted to other women's sexual traits, and it is possible, but less likely, to develop female-female relationships (sexual fluidity hypothesis; Diamond 2009) or to monitor any potential mating competitors (Campbell 1995), but it remains unclear whether similar mechanisms exist in other great apes.

5.5 Conclusion and future perspectives

This study reconfirmed that the hormonal mechanism regulating sexual swellings in bonobo females does not differ from other primate species, including chimpanzees, in that estrogen and progesterone are responsible for swelling onset and detumescence. However, I also confirmed that the resumption of the swelling cycle (including maximal swelling phase) of female bonobos is much earlier (probably, female bonobos are infertile during this early resumed swelling cycle) than that of chimpanzees. Given the large inter- and intra-individual variation in the length of the maximal swelling phase, it is possible that the sexual swellings of bonobos have higher estrogen sensitivity. There are many levels of physiological mechanisms that can account for the high estrogen sensitivity. For example, chimpanzees and bonobos may have some genetic differences in estrogen receptors or other differences in the

tissues involved in sexual swellings. More detailed investigations, including molecular and cytological examinations of estrogen sensitivity in sexual swellings, will help clarify these possibilities.

Poor predictability of ovulation from the onset of maximal swelling in bonobos might be an artifact of variation in the length of the maximal swelling phase across females and cycles. Such variation may result from variation in female reproductive histories (e.g. nursing and early loss), as these events might greatly influence a female's menstrual cycle. However, the poor predictability of ovulation does not seem to be a very big challenge for males, as suggested and discussed in Chapter 3. There is no evidence that the basic hormonal regulatory mechanism of sexual swellings in bonobos differs significantly from other species. Therefore, sexual swellings in female bonobos may also have the same function as that found in other species with (exaggerated) sexual swellings.

It is also possible that male bonobos can recognize subtle size changes within the maximal swelling phases of females. This possibility could not be examined in the present study because of the categorical measure used to distinguish swelling size. Therefore, future studies using digital photography and higher-resolution scales to quantify swelling characteristics is necessary to test this possibility. Longitudinal studies with quantitative measurements of sexual swellings over time will allow for better tests of whether or not f males can discriminate small changes in sexual swelling size or other characteristics such as firmness.

From the present study, it is becoming clearer that male bonobos also compete for mating opportunities as do male chimpanzees, and that males rely on sexual swellings to inform their decisions about mating effort. This also indicates that sexual swellings might be a reliable signal of ovulation – particularly of the females with older infant (over 2.5 years) –, if the variation both within and between females in the length of the maximal swelling phase is also

considered. Male bonobos also compete in particular for access to females during the very narrow fertile window within a menstrual cycle. This mating competition is probably the reason for the increased mating effort of high-ranking males nearer to the fertile periods of females with older infants. This mating competition might also be the reason that low-ranking males usually only accessed copulation with females with younger infants.

The results of Chapter 3 also indicate that males have the ability to determine ovulation in females, regardless of cognitive perception or awareness of ovulation timing within a maximal swelling phase ("males know" hypothesis in Chapter 3). Genetic studies on male paternity in bonobos also support this hypothesis, because paternity is skewed toward high-ranking males (Gerloff et al. 1999; Ishizuka 2017). Therefore, the results presented in Chapter 3 indicate that the efficiency of concealing ovulation via frequent exhibition and long maximal swelling phases is not clear. It is also questionable to assume that the concealing of ovulation decreases male mating competition, because it is also possible that concealing of ovulation can result in severe mating competition by males that are attempting to monopolize reproduction even though they cannot predict ovulation. However, as discussed in section 5.4, low-ranking males can access females with lower probability of conception and this may indeed have an important tension regulation role in bonobo society. Given that the probability of ovulation can hardly be zero during the maximal swelling phase and males might not be able to predict the detumescent day, if males can copulate with females, the prolonged receptivity can indeed play an important social role and influence male mating competitions.

Female bonobos might also use sexual swellings to facilitate female–female bonding. The female–female bonding might play a very important role in the emergence of a peaceful bonobo society, as female–female coalitions against male aggression is highly effective (Parish 1996; Tokuyama and Furuichi 2016). Female–female bonding and coalitionary support among females seems to be one of the most important reasons that female bonobos

have equivocal dominance relationship with males (Furuichi 1997). Although the possibility that prolonged receptivity from frequent and long maximal swelling phases regulates the social tensions between males could not be completely tested in this dissertation, the less restricted sexual activity even among low-ranking males can also be understood as the outcome of the power balance between males and females rather than the cause. If true, this might also explain the huge variation in human sexuality across cultures, particularly among women. It seems that women who have more power, the result of several factors such as higher income, education and social support, suffer less from intimate partner violence (Jewkes 2002). This might also relate to the finding that, in cultures that allow more equality between the sexes, the socio-sexuality (willingness to engage in sexual activity) of women is less restricted (Lippa 2009).

Lastly, if the balance of power between the sexes is important for understanding the social characteristics of bonobos and chimpanzees, it is important to ask what socio-ecological factors have caused this difference in the first place. The answer will not be simple. However, it will be important for us to consider socio-ecological factors, including fruit availability and predation risk, as well as stochastic factors such as founder effects, to better understand bonobo society.

Table 5-1. The results of the linear mixed effect model

Models	predictors	est.	s.e	df	t	*p*
LMM	intercept	13.471	0.610	12	22.087	<0.001
	chimpanzees: bonobos	-2.200	0.863	12	-2.550	0.025

Figure 5-1. Conceptual diagram of maximal swelling and ovulation from parturition to the next conception

Conceptual diagram showing the duration of the maximal swelling phase and ovulation within an individual over time. The horizontal axis represents 1 year and the width of the orange bars represents periods of maximal swelling. The blue bar represents the fertile phase. As infants age, the duration of the maximal swelling phase appears to stabilize. Exceptionally long and short maximal swelling phases might occur in females with young infants.

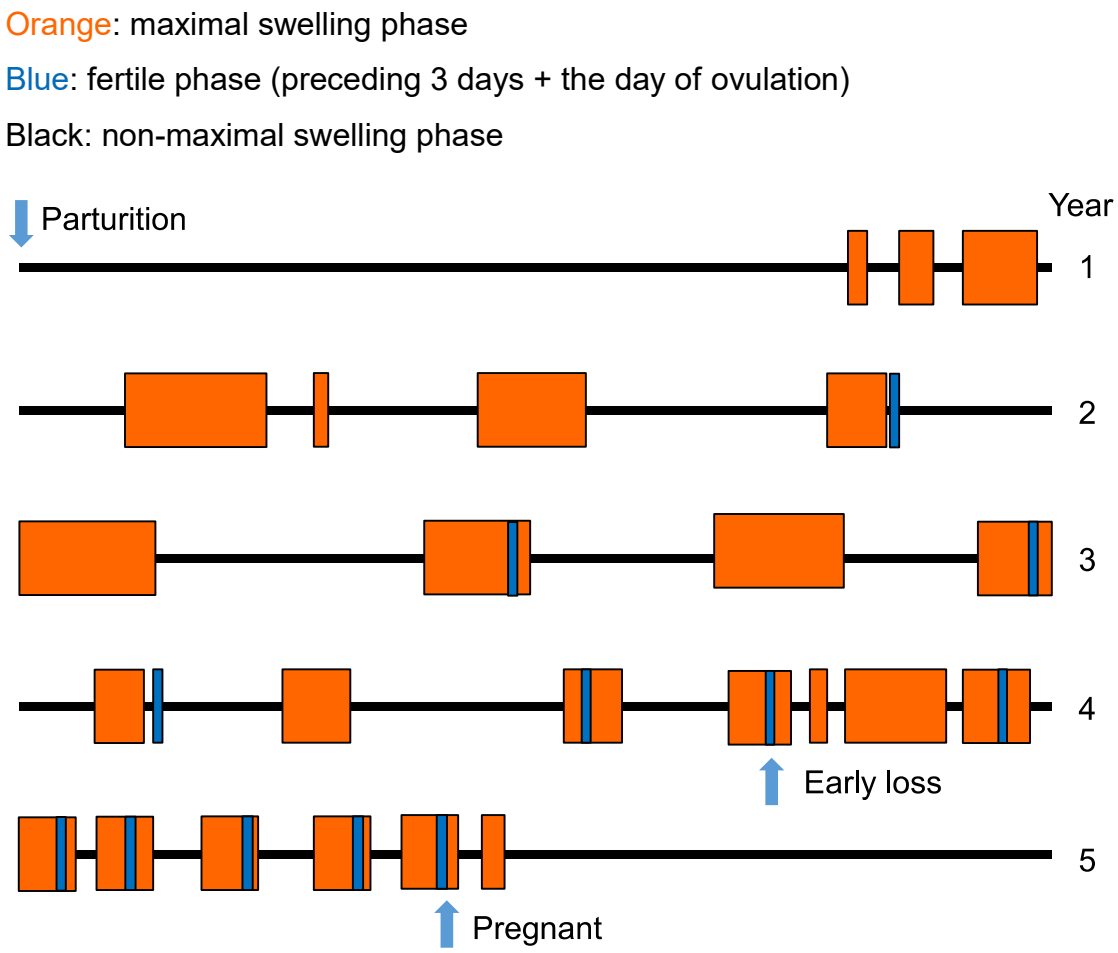
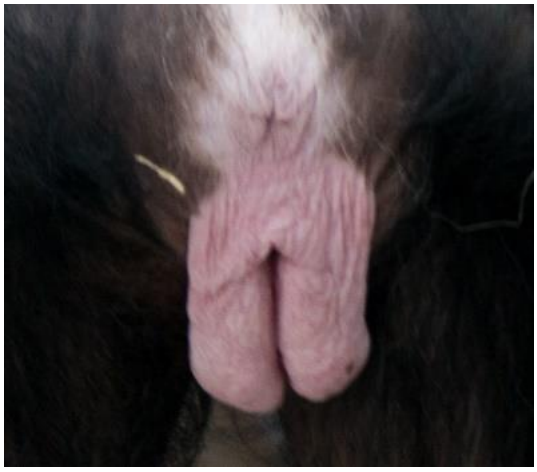


Figure 5-2. Non-swelling phase of bonobos and chimpanzees

Comparison of the minimum size of sexual swellings (non-swelling phase) in female bonobos (a) and chimpanzees (b).

a) 

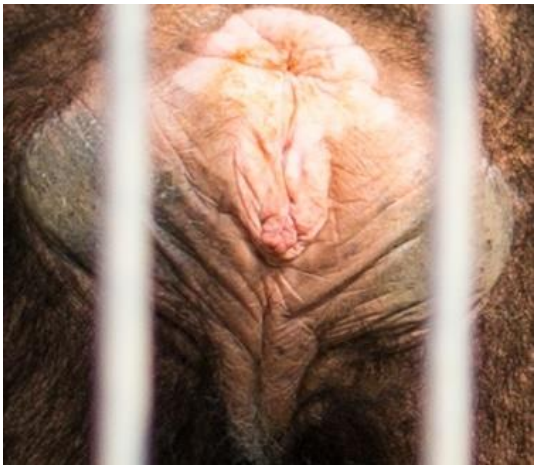
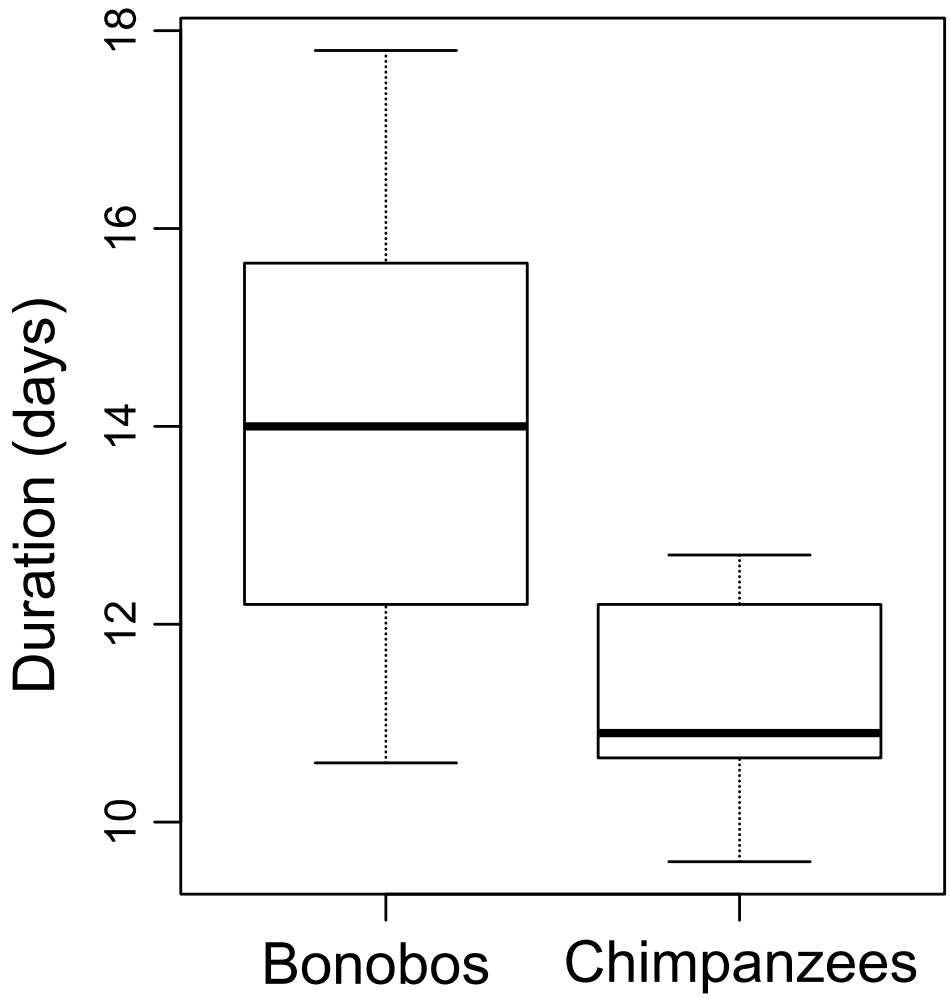
b) 

Figure 5-3. Difference in maximal swelling periods of chimpanzees and bonobos



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