UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

# THE ROLE OF OLFACTION IN SEXUAL INTERACTIONS OF BARBARY MACAQUES (*Macaca sylvanus*) AT AFFENBERG SALEM (GERMANY)

MARIA JORGE ALMEIDA MACHADO

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*To all who dare to chase their dreams*

## O Papel do Olfato nas Interações Sexuais de Macacos-de-Gibraltar (*Macaca sylvanus*) em Affenberg Salem (Alemanha)


### Resumo

A perceção sensorial tem um papel crucial na sobrevivência animal. O olfato desempenha um papel importante nas atividades vitais dos primatas, na procura de comida, reconhecimento de territórios e predadores, e nas interações sociais, como o acasalamento. Várias espécies de macacos, como o macaco-de-Gibraltar, vivem em sociedades com múltiplas fêmeas e machos, e participam em atividade sexual promíscua. A escolha de parceiro pelo macho envolve uma série de componentes sensoriais para detetar a fase de fertilidade da fêmea. As fêmeas exibem uma tumescência proeminente do inchaço anogenital, ligada à aproximação do momento da ovulação, a qual serve como um sinal visual de fertilidade para os machos.

O objetivo do presente estudo foi o de aprofundar a compreensão do uso de sinais olfativos pelos macacos-de-Gibraltar, em particular nas interações sexuais. O *Affenberg Salem*, um recinto ao ar livre lar de quase duzentos macacos-de-Gibraltar, utiliza implantes contracetivos '*Implanon NXT*' em fêmeas para gerir a população. A investigação foi realizada durante o período inicial da época de acasalamento, entre setembro e novembro de 2022. Foram feitas observações de catorze fêmeas-alvo, das quais 7 tinham contracetivo. O principal objetivo da observação foi verificar se os machos recorrem ao olfato nas inspeções sexuais antes das cópulas de forma a selecionar e concentrar seus esforços de acasalamento apenas nas fêmeas férteis (e não tratadas). Durante as observações, foram recolhidos dados sobre todas as ações observadas durante as interações sexuais. Foram tiradas notas e fotos dos inchaços das fêmeas para avaliar a sua influência nas interações e as alterações de tamanho, na tentativa de determinar a fase reprodutiva de cada fêmea. Independentemente de terem ou não contracetivo, todas as fêmeas exibiram padrões de tumescência e detumescência do inchaço anogenital. Além disso, os resultados obtidos demonstram que o estado de contraceção das mesmas não influenciou os diferentes parâmetros de inspeção investigados. Não se verificaram cópulas após inspeções olfativas, tanto em fêmeas tratadas como nas não tratadas.

Foram identificadas limitações do estudo, as quais podem ser utilizadas como ferramenta para melhorar investigações futuras sobre este tema. Entre todas, é importante destacar o período de observação, o qual seria benéfico expandir para o pico da época de acasalamento. Além disso, uma análise detalhada dos perfis hormonais seria útil para compreender e relacionar o comportamento e as ações de inspeção de acordo com a fase reprodutiva das fêmeas.



**Palavras-chave:** Macaco-de-Gibraltar; reprodução para conservação; época de acasalamento; comportamento olfativo; contraceção

**The Role of Olfaction in Sexual Interactions in Barbary Macaques (*Macaca sylvanus*) at Affenberg Salem (Germany)**


## Abstract

Sensorial perception has a vital role in animal survival. Olfaction plays an important role in vital activities of primates, such as finding food, recognizing territories and predators, and in social interactions, like mating. Various macaque species, such as the Barbary macaque, live in multimale-multifemale societies and enroll in promiscuous sexual activity. Male mate choice involves a series of sensorial frameworks to detect the female fertility stage. Females exhibit prominent tumescence of the anogenital swelling, linked to the approach of the moment of ovulation, and which serves as a visual cue of fertility to males.

The aim of the present study was to enhance understanding of the use of olfactory cues by Barbary macaques, particularly in sexual interactions. Affenberg Salem, an outdoor open enclosure home to almost two hundred Barbary macaques, uses contraceptive Implanon NXT implants on females to manage the population. The investigation was conducted during the onset period of the mating season, between September and November 2022. Observations were made of fourteen focal females, of which 7 were contracepted. The main goal of the investigation was to verify if Barbary macaque males use olfactory cues in the inspections of fertility cues prior to copulations to select and focus their mating efforts only on the fertile females (and non-contracepted ones). During observations, data were collected on all actions seen during sexual interactions. Notes and photos were taken of the swellings to assess their influence on the interactions, and their size changes in an attempt to determine the reproductive phase of each female. Irrespective of whether females were contracepted or not, all females showed patterns of tumescence and detumescence of the anogenital swelling. Additionally, in the results, their contraception status did not influence the different parameters of inspections investigated. The data collected did not reveal mating activity after olfactory inspections, in either contracepted or non-contracepted females.

Study limitations can be identified and used as a tool to improve further research on this subject. Among all, it is important to highlight the period of observation, which would be beneficial to expand into the peak of the mating season. Additionally, resources for detailed analysis of the hormonal profiles would be helpful to comprehend and match the individual's behavior and inspection cues according to the precise reproductive stage of the females.



**Keywords:** Barbary macaque; conservation breeding; mating season; olfactory behavior; contraception

## O Papel do Olfato nas Interações Sexuais de Macacos-de-Gibraltar (*Macaca sylvanus*) em Affenberg Salem (Alemanha)

### Resumo Alargado

O macaco-de-Gibraltar é uma espécie ameaçada natural de Argélia, Marrocos e Tunísia, estando extinta neste último país. Trata-se ainda de uma espécie posteriormente introduzida em Gibraltar. Estes primatas vivem em sociedades complexas com grupos de múltiplos machos e fêmeas, que exibem comportamentos sociais intricados, especialmente em relação à reprodução e seleção de parceiros. Esta espécie mostra ainda um sistema de acasalamento poligâmico. Em ambiente natural, mas em cativeiro, como é o caso de *Affenberg Salem*, um recinto ao ar livre na Alemanha, compreender as nuances dessas interações torna-se crucial para a conservação e bem-estar desta, e de outras espécies.

Ao se ter um melhor entendimento dos hábitos, comportamentos e perfil sensorial das diferentes espécies animais, os gestores de parques zoológicos e recintos de vida selvagem podem, através de um enriquecimento ambiental, proporcionar cenários que melhorem o bem-estar dos animais e promovam o estabelecimento de comportamentos naturais. Além disso, este conhecimento pode ser integrado em estratégias de conservação para populações silvestres, ajudando a proteger e preservar as espécies vulneráveis.

A perceção sensorial desempenha um papel fundamental na vida dos animais, auxiliando-os em atividades tão essenciais como a busca de alimento, o reconhecimento de predadores e nas interações sociais. No que respeita ao olfato, a espécie agora estudada é classificada como microsmática (termo que descreve uma capacidade olfativa reduzida – classificação atribuída à ordem dos primatas) e *haplorhini* (um grupo de primatas que se caracteriza pela estrutura simples de nariz que apresenta, comparativamente às espécies pertencentes ao grupo dos *strepsirrhini*).

No contexto das interações sexuais, a deteção de sinais de fertilidade (como a proximidade da ovulação) é particularmente importante. As fêmeas desta espécie de primatas exibem uma tumescência anogenital proeminente perto do momento da ovulação, servindo como um sinal visual de fertilidade para os machos. No entanto, a relevância dos sinais olfativos na determinação da fertilidade e nas interações sexuais ainda não é completamente compreendida.

O presente estudo teve como objetivo avaliar a utilização de sinais olfativos pelos macacos-de-Gibraltar nas suas interações sexuais. Mais especificamente, procurou-se compreender se os machos recorrem à olfação para determinar a fase de potencial fertilidade das fêmeas e se esta estratégia influencia as suas escolhas no momento do acasalamento. O *Affenberg Salem*, parque onde a presente investigação foi conduzida, tem a sua população de macacos-de-Gibraltar organizada em três grupos formados naturalmente: grupos C, F, e H. Cada grupo tem cerca de 50 a 70 indivíduos de todas as idades e de ambos os sexos. Como forma de controlo da população, algumas fêmeas selecionadas receberam implantes

contracetivos '*Implanon NXT*'. Este método contracetivo consiste na aplicação de um implante subcutâneo que liberta continuamente, na corrente sanguínea, etonogestrel, o metabolito ativo do desogestrel, um progestagénio. Dado não ser biodegradável, este implante deve ser removido ou substituído 3 anos após a sua colocação.

A investigação ocorreu durante o início da época de acasalamento, de setembro a novembro de 2022. Foram sujeitas à observação um total de catorze fêmeas, pertencentes aos grupos C e F, entre os 4 e os 9 anos de idade, das quais 7 tinham implantes contracetivos. As observações foram realizadas durante o período de horas de luz disponíveis, entre as 08h30 e as 18h00 - desde o momento em que os indivíduos ficavam ativos até quando subiam às árvores para descansar no final do dia. Cada fêmea focal foi observada em protocolos de 30 minutos, pelo menos 1 manhã (até às 12:30h) e 1 tarde (depois das 12:30h) por semana. A ordem dos protocolos focais a cada semana foi aleatória e em diferentes horas do dia para cada fêmea, sempre tentando distribuir o tempo e o contexto de observação o mais igualmente possível, por fêmea-focal. As fêmeas não eram reobservadas até que todas tivessem as duas observações semanais mencionadas.

No local, em cada protocolo procurou-se registar o comportamento dos machos em relação às fêmeas, com especial atenção para as inspeções olfativas antes das tentativas de cópula. Nomeadamente, foram feitas tabelas personalizadas ao estudo que abordaram os seguintes aspetos: identidade do indivíduo inspetor; quem iniciou a interação (o/a inspetor(a) ou a fêmea inspecionada); se o inchaço da fêmea apresentava sujidade ou sémen; se o/a inspetor(a) olhou, agarrou, e/ou cheirou o inchaço, apenas tocou o inchaço, tocou o inchaço e cheirou a mão; se o inspetor copulou ou apenas montou a fêmea; se houve emissão de sons de cópula; outras informações relevantes; e, se foi efetuada uma gravação. Os dados recolhidos incluíram ainda as fotografias dos inchaços das fêmeas, captadas em cada protocolo, para posterior análise e tentativa de determinar a fase reprodutiva em que cada fêmea estava aquando de cada uma das observações. As interações observadas fora do universo dos protocolos focais do estudo, envolvendo tanto fêmeas focais como não focais, foram também registadas como interações *ad libitum*.

No final do estudo, foi registado um total de 147.3 horas de observações de protocolos. Durante as observações, tanto protocolares como *ad libitum*, envolvendo as fêmeas focais, foram observadas 194 interações, 172 das quais tiveram inspeções associadas e, as restantes 22, foram cópulas ou montas que não tiveram uma inspeção prévia.

Foi realizada uma análise estatística com recurso ao programa R a fim de perceber a relação entre variáveis observadas e consideradas de maior relevância. Foram efetuados dois conjuntos de testes: um para comparar os efeitos da contraceção nas fêmeas e outro para correlacionar o tamanho mínimo dos inchaços com diferentes parâmetros. Quanto à análise de impacto da contraceção, apenas um teste revelou uma diferença significativa entre as variáveis no caso: observaram-se mais inspeções com atividade olfativa quando não havia sujidade no inchaço das fêmeas. Quanto ao estudo de correlação do tamanho mínimo do inchaço, apenas dois testes apresentaram resultados significativos: à medida que o tamanho mínimo do inchaço aumenta, a taxa de inspeções com recurso ao olfato diminui; e as fêmeas que começam a aumentar o volume do inchaço mais tardiamente são as que apresentam um menor inchaço mínimo.

Os resultados mostraram ainda que, independentemente de terem ou não implantes contracetivos, todas as fêmeas exibiram padrões de tumescência e detumescência do inchaço anogenital. Adicionalmente, o estado contracetivo não influenciou significativamente os diferentes parâmetros de inspeção investigados. Em nenhuma das observações foram registadas cópulas após inspeções olfativas, tanto nas fêmeas tratadas, quanto nas não tratadas.

Assim, estes resultados sugerem que, apesar de por vezes subtis, os sinais olfativos estão presentes. A tumescência anogenital fornece um indicador visual claro que pode ser rapidamente avaliado pelos machos, facilitando as decisões de acasalamento mais imediatas.

Uma das principais limitações deste estudo foi o período relativamente curto de observação, que cobriu apenas o início da época de acasalamento. Observações durante o pico da época de acasalamento poderiam fornecer uma visão mais completa das interações sexuais e do uso da inspeção olfativa. Além disso, a ausência de uma análise hormonal detalhada limitou a compreensão das correlações entre os sinais olfativos e o estado reprodutivo das fêmeas. De forma a contornar essas limitações, futuros estudos deverão alongar o período de observação e incluir análises hormonais detalhadas. Isso permitiria uma compreensão mais aprofundada das interações entre sinais visuais e olfativos, bem como como estes poderão influenciar o comportamento de acasalamento dos machos.

Em conclusão, o presente estudo pode contribuir significativamente para a compreensão das interações sexuais dos macacos-de-Gibraltar, destacando a predominância dos sinais visuais na escolha das parceiras pelos machos. A investigação no *Affenberg Salem* mostrou que, embora os sinais olfativos estejam presentes, eles não desempenham um papel decisivo nas opções de acasalamento dos machos. Estas descobertas podem ser muito importantes na gestão e conservação da espécie, especialmente quando em cativeiro.

**Palavras-chave:** Macaco-de-Gibraltar; reprodução para conservação; época de acasalamento; comportamento olfativo; contraceção

## Comunicação em Congresso

Parte dos dados recolhidos no presente estudo foram apresentados numa comunicação em congresso, em formato de *poster* (anexo 1), na *International Behavioral Conference 2023*, após revisão por pares, com o título:

Weiß B.M., Simon M., Ritter N., Machado M.J.A., Widdig A. (2023) The role of olfaction in social interactions of Barbary macaques (*Macaca sylvanus*). Conference: Behaviour 2023. August $14^{th}$-$20^{th}$, Bielefeld, Germany.

## Communications in congress

Some of the data collected during the observations of the present study were presented in a congress, as a poster (appendix 1), at the International Behavioral Conference 2023, after acceptance by peer review, with the title:

Weiß B.M., Simon M., Ritter N., Machado M.J.A., Widdig A. (2023) The role of olfaction in social interactions of Barbary macaques (*Macaca sylvanus*). Conference: Behaviour 2023. August $14^{th}$-$20^{th}$, Bielefeld, Germany.

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## List of Abbreviations

DFG: German Research Foundation

CDI: International Dressage Competition

EAZA: European Association of Zoos and Aquaria

C: Contracepted

NC: Non-Contracepted

ND: No Data

Obs: observations

NA: Not Applied

EVA: Ethylene Vinyl Acetate

E:P : estrogen:progestogen

Nº: number

JD: Julian Dates

$\rho$ : Spearman rank coefficient correlation

$r$ : Pearson's rank coefficient correlation

MPA: medroxyprogesterone acetate

GC-MS: gas chromatography-mass spectrometry

SPME: solid-phase microextraction

## 1. Internship Activities

Within the study plan of the Integrated Master's Degree in Veterinary Medicine at the Faculty of Veterinary Medicine of the University of Lisbon, the curricular internship was carried out in the area of animal production and food security, more specifically sexual behavior in an observational research context. This work was developed at Affenberg Salem, a zoological park in Salem, Germany, which is home to Barbary macaques. The on-site work had a duration of 2 months and 1 week, from September 5 to November 11, 2022, followed by 9 months of off-site work.

The activities were developed under the guidance of Doctor Brigitte M. Schlögl, a biologist who is part of the Behavioral Ecology Research Group at the Institute of Biology of the Faculty of Life Sciences of the University of Leipzig, Germany. The study developed during this curricular internship was part of Dr. Brigitte's DFG (German Research Foundation) grant "Olfactory cognition of social relationships in primates" and focused on the Barbary macaque's olfactory cues in sexual interactions.

At Affenberg Salem, the work began by learning to identify every individual, focusing on two of the three social groups that integrated the study developed. Furthermore, a key skill developed was to learn how to walk and react among the animals so that there was minimal human interference with the data collected, as well as safety on-site. Twenty days after the beginning of the internship, data collection began, on September 25, 2022. The data collected were based on behavior observation of parameters that the tutor and trainee discussed and agreed to be of more relevance to the aim of the research. When it was needed, the trainee helped with other activities non-directly related to the study at hand, like helping to catch individuals and notify regarding injured animals. In the field, the hours of work were dependent on the hours of sunlight available, rounding 8 hours per day at the beginning of observations and ending on an average of 6 hours of work per day. When the sun went down, the work continued indoors, organizing the data collected during the respective day. The park closes for visitors each year at the beginning of November, and its director was not in the park after November 11, 2022, which forced the observations to end on that last date for safety reasons for the trainee.

After data collection, an off-site work of about 9 months began. During this period, the activities developed were based on working with the data collected, organizing it, selecting the relevant information, and analyzing it using the R program to find conclusions for the question and prediction proposed by the tutor and trainee.

As far as extracurricular activities go, during the period off-site, the trainee also aimed to develop some clinical skills, participating in equine clinical cases of DVM Joana Matos from Clínica Veterinária JM in Trofa, Porto, Portugal. The clinical activities participated in included:

- Helping on the castrations of a donkey and a horse;
- Taking blood samples and administrating different medications and dewormings;

- Helping on a case of uterine infection in a mare, on its diagnoses, pharmacological treatment, and Caslick's suture including post-procedural care;
- Helping on an artificial insemination case of a mare, doing ultrasounds pre-insemination and to confirm gestation, administrating medications, and assisting during the procedure;
- Helping in orthodontal cases, assisting in the removal of wolf teeth and in the floating of teeth.

In horse clinic extracurricular work, the trainee has also assisted DVM Bruno Miranda during the horse veterinary inspection at the CDI 3* (International Dressage Competition) of Companhia das Lezírias in April 2024.

Additionally, as an extracurricular occupation, the trainee is also developing knowledge on an EAZA (European Association of Zoos and Aquaria) learning online course. This said course is on the topic 'Reproductive management of zoo and free-ranging species'.

## 2. Introduction

In the wild, animals need to rely on their different senses to survive. Out of all the senses, olfaction takes, across mammals, an essential role in several fundamental contexts, like in social interactions (Simon et al. 2023). Among primates, as far as evolution goes, optic convergence and stereoscopic vision have evolved, linked to a reduction in the relative size of the olfactory system (Kay 2018). The use of olfaction by non-human primates has been well recorded, but studies have focused more on strepsirrhine and platyrrhine primates than on catarrhines such as Barbary macaques (Simon et al. 2023). This is due to the assumption that olfaction does not play such an important role in the lives of animals from taxa with well-developed optical skills (Barton 2006). However, it is known that the survival of animals is highly dependent on the sense of smell, called olfaction. Primates use olfactory cues to find food, avoid danger, recognize territories, and in social behaviors like mating (Niimura and Nei 2006). Strepsirrhine primates often have a nocturnal lifestyle and, therefore, have less specific visual capacities and show an enhanced olfactory apparatus. For this reason, these taxa have been the focus of more studies on olfactory behavior within the non-human primates' universe than other taxa (Barton 2006). However, in the case of catarrhine primates, such as Barbary macaques, classified as "microsmatic" species (Smith and Bhatnagar 2004), not many studies have been conducted to understand the importance and use of olfaction.

Research on olive baboons (*Papio anubis*, a non-human primate from the same phylogenetic family as the Barbary macaque) has revealed that their sexual communication consists of multiple sensorial cues and signals. These multimodal components act in concert to enable males to assess the probable timing of ovulation in females. The more combined use of sensorial modalities, the more information males may obtain to make a well informed decision in their mate choice and decrease the expenses associated with mating, such as sperm production (Rigaill et al. 2013). A previous study by Young et al. (2013) also found that Barbary macaque males do a close inspection of females' anogenital swellings to determine their reproductive state. That study suggested that "males may be able to pick up on further hormonal cues given that the inspection rate increased towards the fertile phase" (Young et al. 2013).

The Barbary macaque is the only macaque species from northern Africa. Almost fifty years ago, it was reported that they could be naturally found in fragmented groups in which populations were jeopardized due to extensive attacks on the forests (Taub 1977). Consequently, natural populations have been constantly decreasing over time, making breeding in open enclosures an important tool for this species conservation.

The present study aimed to assess if contraception affects sexual interactions and whether such effects might be mediated by olfaction. This is a topic of interest to improve the population management of this focal species, the Barbary macaque. A better understanding of their social and mating behavior is essential to provide living conditions according to their needs and to offer an enclosure that ensures the best possible environmental conditions and husbandry regime. This understanding is possible as the study site, Affenberg Salem, Germany, has living conditions very similar to those found in the Barbary macaques' natural habitat. Despite some study limitations, this research may serve as a pilot study for further research on this subject.

## 3. Literature Review

### 3.1. Summary Review of the Barbary Macaque (*Macaca sylvanus*)

According to the IUCN Red List of Threatened Species, the Barbary Macaque, *Macaca sylvanus*, belongs to the kingdom Animalia, phylum Chordata, class Mammalia, order Primates (Fooden 2007), infraorder Catarrhini (Fleagle 2013a), and family Cercopithecidae. This Old-World monkey is the only macaque species found outside of Asia in which the tail is completely absent or reduced to a boneless vestigial tail (Fooden 2007).

In the wild, Barbary macaques occur from sea level to 2.600 m above sea level, which means that they live in bioclimates from upper semi-arid to upper sub-humid (Cuzin 2003). This macaque species is native and extant (resident) from Algeria and Morocco, native and extinct from Tunisia, and extant and introduced (resident) in Gibraltar (The IUCN Red List of Threatened Species 2020). This species has seen its population decline by 50% over the last 3 generations (24 years), qualifying it as Endangered under criterion A2bcd, with predictions that this decline will continue in the future, signaling the current population trend as decreasing (The IUCN Red List of Threatened Species 2020). This concern made breeding in captivity an urgent matter.

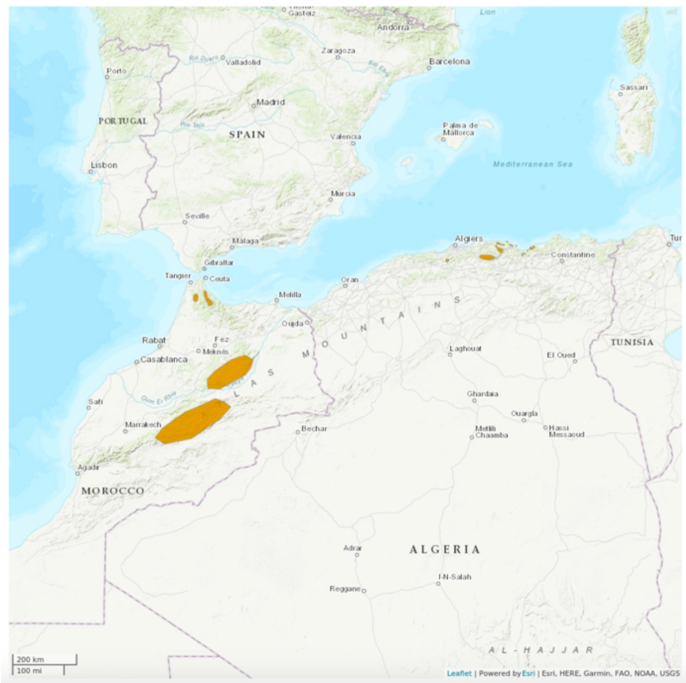



**Figure 1: Distribution map of wild Barbary macaque populations compiled by Janette Wallis and IUCN (International Union for Conservation of Nature) 2020**

Legend: - Extant(resident)

## 3.2. Barbary macaque Morphology, Anatomy, Physiology, and Reproductive Biology

### 3.2.1. Reproductive Anatomy

Across the primate order, a hallmark feature of their reproductive biology aligns with the mammalian norm. This entails internal fertilization of the egg, followed by embryonic development occurring within the female's uterine environment over an extended gestational period, culminating in live birth (Fleagle 2013b). Barbary macaques have a mean gestation period of 164.7 days (Küster and Paul 1984).

The same applies to the anatomic structures – figure 2. Males exhibit paired testicles suspended in the scrotum at the caudal end of the anterior abdominal wall. The scrotum is usually located behind the penis, but it can be found in front of it, depending on the species. Species-specific is also the size of the testes, which are correlated with the respective mating system (Fleagle 2013b): males showing larger testes live in polyandrous groups with considerable male-male competition, like the Barbary macaque societies; while males from single-male and monogamous groups with low intrasexual competition show smaller testes (Fleagle 2013c). Most non-human male primates have a bone, named baculum, inside the penis (Fleagle 2013b).

Nonhuman-primate females possess paired ovaries and paired fallopian (or uterine) tubes, which are laterally projecting from the midline uterus towards the ovaries. The size of these structures is variable between primate species. Below the uterus, there is the vagina opening onto the perineum, the spot where is evident the external genitalia. The external genitalia of female primates typically comprises bilateral labia adjacent to the vaginal orifice, alongside the clitoris situated anteriorly to the vagina. Furthermore, numerous female primates exhibit sexual skin regions surrounding their external genitalia, exhibiting chromatic and dimensional variations throughout their reproductive cycle. In some species, such as Barbary macaques, baboons, and chimpanzees, these sexual skin swellings are large and serve as a prominent display of an individual's reproductive status (Fleagle 2013b).

The development of the fetus within the mother's womb differs between primate species. Macaques and tarsiers form a hemochorial placenta: confined into one or 2 placental disks that invade the uterus wall, providing a close interchange of fetal and maternal blood supplies – figure 3 (Fleagle 2013b).

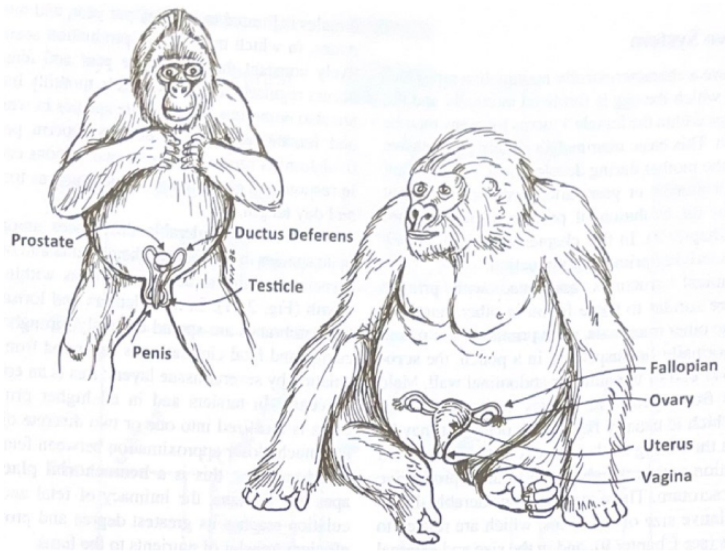



**Figure 3: Primate male and female reproductive structures and respective legend, using gorillas as an example (from Fleagle 2013b)**

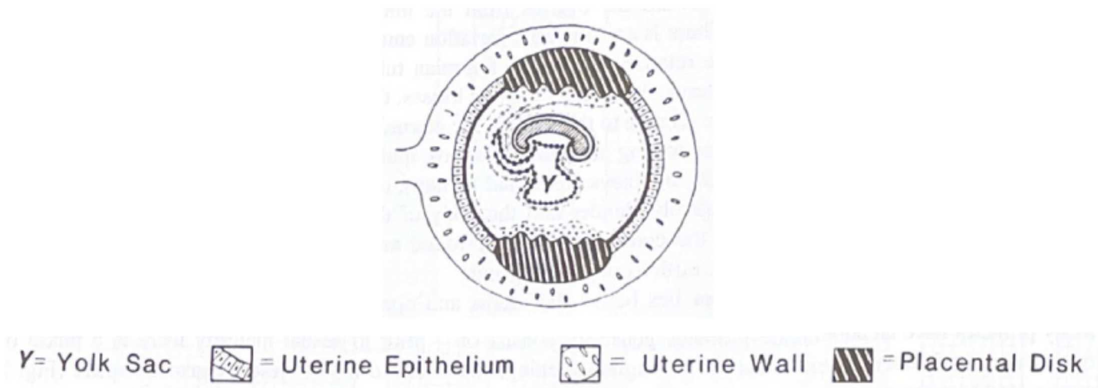



**Figure 2: Fetal membranes in macaques with the respective legend (from Fleagle 2013b)**

## 3.2.2. Sexual Maturation

The timing of sexual maturation of females in semi-natural enclosures is similar to that registered in wild populations (Turckheim and Merz 1984). A report in the Salem enclosure by Paul and Kuester (in Fooden 2005) showed that females could have their first young at 4 years of age. An analysis made in 1993 of the reproductive history of the females from Affenberg Salem showed that *M. sylvanus* females' fertility suffers a decline from prime age (7 to 12 years of age) to mid-age (13 to 19 years of age), and from mid-age to old age (20 to 25 years of age). Older mothers weaned their infants later and showed more maternal investment than younger mothers, which translated into highest survival rate of offspring of old females and longer interbirth intervals. Three or 4 years after the birth of the last infant, reproduction cycling invariably ceased, although it was found common that the post reproductive life span was more than 5 years (Paul et al. 1993).

### 3.2.3. Female Sexual Cycles

Previous observations reported that, during the mating season, *M. sylvanus* females show coordinated reproductive changes of sexual skin swelling, menstruation, and estrus behavior. In this species there is a clear degree of reproductive seasonality (Möhle et al. 2005). The sexual skin swelling refers to two perineal areas (one around the anus and the other bilateral to the vulva cleft) and it shows "pronounced cyclical changes during the mating season and early pregnancy" (Möhle et al. 2005). These swellings' sizes vary with age, as the young individuals show a bigger swelling size increase, and the old ones exhibit a not-so-marked evolution. Females mate with different males and show initiative in contact with mating partners. When pregnant, females continue to copulate with males (Turckheim and Merz 1984).

The swelling size is related to the estrogen: progesterone (E:P) ratio throughout the follicular phase (Möhle et al., 2005). Prominent tumescence is linked to an increase in the fecal estrogen level, while detumescence is associated with an increase of fecal progesterone level. Ovulation occurs during the maximum swelling stage, but its precise timing is variable (Möhle et al. 2005).

During the mating season, Möhle et al. (2005) reported that the 2 nulliparous females in their study had earlier onset of the swelling increase than the primi- and multiparous females. This led them to believe that parity might be a factor that influences the onset of swelling increase.

In the postconception period, an increase in swelling has also been linked with vaginal bleeding between days 18 to 30 of gestation (Möhle et al. 2005). This is preceded by a rise in the E:P ratio values within the ones found in the mid-to-late follicular phase. Measurements taken by Möhle et al. (2005) show that these early gestation swellings were slightly yet significantly smaller than the ones registered during the conception cycle. A subsequent decrease in estrogen fecal concentration, as there is an increase in progesterone levels, leads to an E:P ratio back to low baseline levels and, in consequence, a decrease in swelling size. Therefore, in *M. sylvanus*, postconception anogenital swellings seem to be influenced by hormonal control mechanisms like the ones that are registered during the ovulatory cycle (Möhle et al. 2005).

Fecal estrogen and progesterone profiles collected in Gibraltar for the referred study by Möhle et al. (2005) indicate that Barbary macaque females are physiologically infertile outside the mating periods. They found that conceptions and ovarian cyclicity were synchronized and the ovarian function was influenced by seasonal or social factors. Additionally, since the nulliparous females observed in the study were also acyclic outside the mating season, suckling infants did not appear to influence this ovarian acyclicity. The swelling size was not correlated with body mass, nor was a pronounced age effect found (Möhle et al. 2005).

### 3.2.4. Copulation

The multimale, multifemale organization of macaque societies gives the genus a complex reproductive strategy. Therefore, macaques show a mating system described as polygynous, polygamous, and promiscuous (Dixson 1998;Soltis 2004). Like observed in other macaque species, Barbary macaque males usually mount females dorsoventrally by gripping their trunks and shanks with, respectively, their hands and feet (Fooden and Aimi 2005 in Fooden 2007).

In combination with copulation, Old World primates' females produce intense vocalizations, known as copulation calls, which are related to changes in the reproductive hormones' concentrations (Pfefferle et al. 2011). A study conducted by Pfefferle et al. in 2011 concluded that periods of elevated levels of estrogen (more specifically the peak that precedes ovulation during the conception cycle and the peak of the postconceptional period) were associated with more intense mating activity and more frequent copulation calls. However, the correlation between the calls and the endocrinal parameters was most marked during the postconception rather than during the conception cycle.

The two reproductive hormones have different modulating effects on call characteristics depending on the reproductive stage. While the estrogens in the postconception period had a positive influence on call frequency and duration, an increase in progesterone levels led to a reduction in the same call parameters (Pfefferle et al. 2011).

### 3.2.5. Olfaction

Odors are comprised of a mixture of different molecules. The different odor molecules are detected and distinguished by proteins known as olfactory receptors (OR) encoded in the animal genome (Niimura and Nei 2006). However, there is evidence that different species have different olfactory sensitivity, and, therefore, a classification has been made using the terms "microsmatic" and "macrosmatic". Respectively, they refer to species with reduced or enlarged levels of olfactory function (Smith and Bhatnagar 2004). Primates have olfactory bulbs (the neural structure in the forebrain involved in olfaction) proportionately smaller in absolute size compared to other mammals. Furthermore, primates also have proportionally less surface area in the nasal cavity covered with olfactory epithelium (the region that contains odor receptor cells) than other mammals. For those reasons, this animal order has been classified as microsmatic (Smith and Bhatnagar 2004). However, more recent studies focusing on the different primate species have questioned this view and proposed a different classification to this animal order according to the features of their nostril shape: haplorhines, which means "simple nose", and strepsirrhines, which means "curved nose" (Niimura et al. 2018). These groups have, in terms of relative size, respectively, smaller and bigger olfactory bulbs (Smith et al. 2007). Old World Monkeys, such as Barbary macaques, are included in the haplorhines group (Niimura et al. 2018).

## 3.3. Social Organization

Social organisms must synchronize behaviors and coordinate collective actions to exploit the advantages conferred by group living arrangements (Seltmann et al. 2013). Macaque groups show complex social relations, all living in relatively large multi-male multi-female troops (Fleagle 2013a).

Typically, within the social structures of most primates, there is territorial behavior. The species belonging to the family Cercopithecidae, like *Macaca sylvanus*, show a "troop type" social unit which is constituted by a matrilineal genealogical group along with one or more adult males (Itani 1977). These matrilineal organized groups that characterize macaque species show same-sex substructures of social units (Bartecki 1986). In this group-living society design, individuals benefit through the establishment of a vast network of social relationships (Sueur et al. 2011).

Through systematic observation and analysis, several key findings emerged on Barbary macaques' social interactions within the group from a study conducted in 1999 by Kümmerl and Martin (published in 2008). In the realm of papionin primates (a tribe of Old World Monkeys), the Barbary macaque (*Macaca sylvanus*) exhibits the most profound engagement in interactions between infants and group members other than the mother. These interactions manifest in, on one hand enduring dyadic engagements between a handler and an infant, and on the other hand brief triadic interactions involving an infant between two handlers. All males, females, and juveniles partake extensively in triadic interactions, utilizing infants born from above-average related females (Kümmerli and Martin 2008).

In the context of hierarchical structure, rank of males and females Barbary macaques are dependent on different factors. Significantly contrasting with other 2 macaque species (rhesus and Japanese macaques), the social dynamics of Barbary macaques unveil an early establishment of male dominance over females. Remarkably, male Barbary macaques assert dominance over females of the same age as early as within the first year of life. A shift in dominance emerges during the second year of life, as males begin to surpass older, unrelated females in rank. By the age of 5 to 6 years, male Barbary macaques have unequivocally surpassed all females in the hierarchy, consolidating their hierarchical ascendancy within the social structure of the species. (Kuester and Paul 1988).

Among female macaques, maternal rank predominantly influences female rank acquisition, albeit subject to influence by age and size discrepancies among individuals across different familial units. While instances of mother-daughter rank reversal are infrequent, it was observed that aging, post-reproductive matriarchs consistently lost their ranks to their adult daughters. Notably, unlike findings from previous studies on macaques, the phenomenon of younger sisters surpassing their older counterparts in rank is uncommon (Paul and Kuester 1987). However, unlike the rigid age-inversed hierarchy observed in adult sisters of rhesus and Japanese macaques, no such genealogical pattern was evident among the studied population of Barbary macaques (Paul and Kuester 1987).

Similar to those of rhesus and Japanese macaques, the establishment of rank relations among males of the same age occurs early in life, with hierarchies among peers typically following a linear structure, persisting with remarkable stability over extended periods. Despite this general pattern, instances of rank reversals within peer cohorts have been observed, even though infrequently, suggesting a normative yet sporadic phenomenon (Kuester and Paul 1988). Comparatively, Barbary macaques exhibit lower frequencies and lesser extents of hierarchy overlap when contrasted with their rhesus and Japanese counterparts. Maternal rank exerts a diminished influence on male rank, with no discernible cohort exhibiting a complete alignment of male rank order with maternal status alone; instead, male age, indicative of the timing of birth within the reproductive season, emerges as a factor of comparable significance. Interestingly, even the offspring of the highest-ranking females display no deviation from this trend (Kuester and Paul 1988). Circumstances that typically disrupt the normative rank acquisition process, such as early maternal loss observed in rhesus and Japanese macaques, do not account for the weaker maternal influence on male rank observed in Barbary macaques. The timing of male birth confers a distinct advantage, particularly for offspring of low-ranking females, although birth timing is contingent upon recent reproductive histories, with early births often following periods of sterility or infant loss (Kuester and Paul 1988).

Notably, unlike in rhesus and Japanese macaques, male Barbary macaques hold a significant influence over the rank of their younger brothers, particularly those born to high-ranking mothers, who tend to have larger sibling cohorts, thereby enhancing their access to support networks. However, the positive effects of maternal and fraternal support are mitigated by the negative consequences associated with late birth, even for older brothers hailing from high-ranking lineages within their respective cohorts (Kuester and Paul 1988).

### 3.4. Barbary Macaques in Outdoor Open Enclosures

Four large parks are working together dedicated to Barbary macaques open: 2 in France - “La Montagne des Singes” and “La Foret des Singes” -, 1 in Germany - “Affenberg Salem” -, and 1 in England - “Trentham Monkey Forest”. These colonies were created from an initial group of wild-caught Barbary Macaques from Morocco. The Barbary macaques are characterized by their low aggressiveness and their ability to thrive in highly seasonal environments, like the ones found in North-western Europe, where the enclosures are located. Here there are typical cold snowy winters and high annual sunshine, which is why they can prosper in these enclosures’ conditions (Turckheim and Merz 1984).

In the parks, the animals can roam freely within outdoor open enclosures all year round. The spaces consist of open forests kept as natural as possible, with no human-made shelters or housing, where the animals live on both natural and provided food and water. They are protected by a fence with electrified wires at the top and a wide clear area around the enclosures so that the monkeys cannot escape by climbing and jumping the trees (Turckheim and Merz 1984). Furthermore, at Affenberg Salem, human contact and interference are kept to a minimum, and the monkeys are discouraged from touching visitors and staying in the visitors' path. There are areas where the animals can go and stay out of sight and hear from people. It is usual to find females with infants and shy individuals in these more private places. The parks are closed to visitors from November to March (Turckheim and Merz 1984).

The aim of these parks was to create a tourist attraction exhibiting monkeys in near natural habitat conditions to the public so that visitors can see naturally formed social groups of wild monkeys at close range in harmony with their habitat. The enclosures serve an educational purpose, being used by schools and providing material for teaching programs, as well as a valuable center for scientific studies on the Barbary macaques' biology and behavior and complement research on wild populations (Turckheim and Merz 1984).

### 3.4.1. Conservation Breeding

Most macaque species typically give birth to one offspring each year (Fleagle 2013a). The breeding at the parks, as well as in the wild, is seasonal. Mating occurs in autumn and winter and births in spring and early summer. The first copulations start at the end of the summer, and during October and November, sexual behavior is more active. According to Fooden (2007), the peak of the mating season is from November to January. After that, sexual activity is strongly reduced. In the Salem enclosure, a research conducted during the breeding season 1982/83 showed that the age, parity, and reproductive history of the females influenced the onset of the sexual activity of each individual and, consequently, the overall media of the onset of the mating period of the group. Thus, younger females and females with infants born in the year before conceived later in the year than older females (>10 years old) and parous females not raising an infant from the year before, respectively (Küster & Paul 1984).

### 3.4.2. Female Contraception: Implanon NXT

The strategic planning of a female organism's reproductive trajectory to sustain fertility necessitates the integration of her genetic fitness, ecological carrying capacity for offspring, and species-specific reproductive physiology. Modalities for regulating offspring production over her lifetime and modulating inter-birth intervals encompass strategies such as separation of the sexes and contraception. However, the selection of a suitable approach is complex, owing to pronounced interspecies variations in responsiveness to diverse contraceptive methodologies available for implementation in wildlife management contexts (Penfold et al. 2014).

To control the population size in outdoor open enclosures, the Barbary macaques' parks mentioned above resort to female contraception. The contraception used at Affenberg Salem, Germany, the place of the study, is a subdermal progestogen-only implant called Implanon. It consists of a rod of 4 cm in length and 2 mm in diameter of an ethylene vinyl acetate (EVA) matrix covered by a rate-limiting EVA membrane. This device has dispersed 68 mg of the desogestrel active metabolite etonogestrel, which is a progestogen. The implant must be removed after three years of insertion, as it is non-biodegradable. Initially, after implantation, Implanon releases continuously into the bloodstream 60-70 μg/day of etenogestrel, descending to about 40 μg/day by the end of the first year, to 30-34 μg/day by the end of the second year, and to 25-30 μg/day by the end of the third year of this contraception. This implant shows a bioavailability close to 100% and a clearance of about 7.5 l/h. Hence, as these values remain steady, etonogestrel does not show to accumulate in extended use. Overall, this contraception method has a similar effect as a daily progestogen oral contraceptive, which exposes the individual to a daily low dose of progestogen and has a low rate of adverse effects (Coelingh Bennink 2000).

In what comes to the contraceptive effects, Implanon demonstrates direct and indirect effects: the first through endometrial progestin target sites on the endometrium, and the second via suppression of the hypothalamic-pituitary-ovarian axis (Varma and Mascarenhas 2001). Thus, this implant's efficiency is mainly due to the inhibition of ovulation and, secondarily, due to changes in the cervical mucus, which leads to impairment of sperm movement and endometrial implantation.

A study conducted in 1995 on a group of Barbary macaque females living at the Affenberg Salem enclosure, Germany, implanted with levonorgestrel contraceptive (a synthetic version of the natural hormone progesterone, such as etonogestrel) registered a fecal steroid concentration with elevated estradiol and reduced testosterone levels compared to non-contracepted females (Wallner et al. 2007). Within the contracepted group, the excreted progesterone levels also showed a higher variation. Regarding the anogenital swellings, in the same study, implanted females exhibited either reduced swellings with a high rate of progesterone excretion or larger swellings with a low rate of progesterone levels. The first case could be due to luteinized unruptured follicles present in the ovaries. As for the second case, they could be females with persistent follicles. Therefore, among the implanted females, the perineal swelling size seems to be regulated by the progesterone concentration (Wallner et al. 2007).

Behavior-wise, there is another study published in 2016 that observed contracepted with Implanon and non-contracepted adult Barbary macaque females living at the Trentham Monkey Forest enclosure, United Kingdom. This investigation concluded that the contraceptive Implanon has several behavioral effects on free-ranging females. Contracepted individuals showed higher rates of anxiety, which is reflected in the bigger frequency of selfscratching and self-grooming. It was registered less giving and more receiving grooming in these females, as well as more traveling and less resting times, but no difference was noticed in foraging times. In addition, individuals implanted with Implanon showed more aggression towards conspecifics (Maijer and Semple 2016). However, no article was found evaluating the effects of contraception on females' sexual behaviors.

## 4. Experimental Work

### 4.1. Objectives

The focus of the present study was to observe how male Barbary macaques might use olfaction during sexual interactions. Since contraception is used to control the population in the females of the groups observed, it was relevant to assess if contraception influenced the individuals' sexual interactions. Furthermore, if such changes were observed, investigate if males would use olfactory cues to gain information on the females' reproductive state, differentiating females with and without contraceptive implants.

It was expected that males would rely on olfaction during sexual inspections to determine which females would be at a fertile phase (close to ovulation) and, therefore, that those individuals would be the ones non-contracepted. Following the previous assumption, males would invest in mating with fertile females instead of spending their time and energy on females with contraception or in a non-fertile reproductive stage with whom, therefore, they would not have a successful mating interaction. Thus, it was envisioned that more sexual interactions involving non-contracepted females than contracepted ones would be recorded. It was also presumed that contracepted females would show less libido than non-contracepted ones during the estrus cycle, in the form of approaching the male and allowing him to explore and engage with them.

The observations also explored whether other senses, like visual cues (for example, seeing dirt), might module the way in which males explore the females' anogenital swellings, their main tool of evaluating the females' reproductive stage. For this topic, the observer looked at the approach (looking, touching, smelling) of the males upon the encounter of different conditions of the females' anogenital swelling (if it was clean, had dirt or even semen, and in what degree).

### 4.2. Material and Methods

#### 4.2.1. Study Site and Subjects

The study was conducted at Affenberg Salem, a park located in Salem, close to Lake Konstanz, Germany, home to almost 200 Barbary macaques living freely in a 50-acre fenced forest. At this location, the monkeys have climate conditions similar to those they would find in their natural habitat and, therefore, live all year outside, and maintain their natural behavior. Visitors have access to a restricted path from March to November that covers around one-third of the enclosure, while the Barbary macaques roam freely around the entire grounds. The park is closed to visitors from November to March (Affenberg Salem website, accessed in October 2023: *https://www.affenberg-salem.de/en/barbary-macaques/*). The monkeys live on natural food and insects they find throughout the forest, daily fruit and vegetable supplements provided, and wheat distributed around the feeding areas. Water is available in several water reservoirs *ad libitum* and in the forest in the form of ponds, water on leaves, and tree holes.

Affenberg Salem has three naturally formed groups of individuals: groups C, F, and H. Each group is formed of 50 to 70 individuals of all age classes and both sexes. Every monkey is identifiable by its natural markings and a tattoo on their upper left inner thigh. This tattoo contains their identification code which starts with a letter (corresponding to their year of birth) followed by a number. To manage population size, some females receive contraceptive hormonal implants (Implanon NXT).

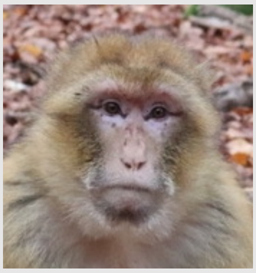
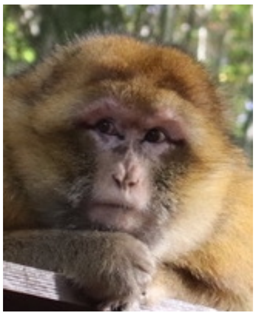
This study was purely observational and in accordance with the legal requirements of Germany and Affenberg Salem internal rules.

### 4.2.2. Behavior Observation

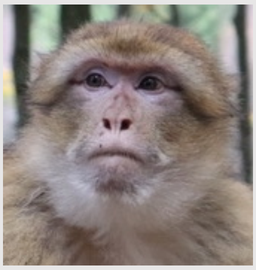
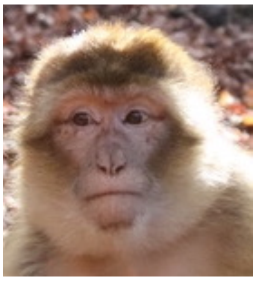
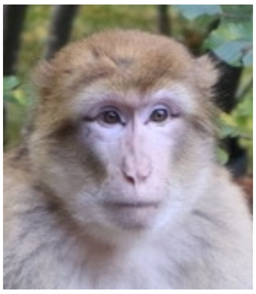
The study was conducted from September 25, 2022, until November 11, 2022. Observations were conducted during the available daylight hours between 08:30 a.m. and 06:00 p.m., from the time the individuals became active to when they climbed the trees to rest at the end of the day. The earliest observation was a focal protocol that started at 08h:32m:00s and the latest observation was another focal protocol that ended at 17h:44m:45s. According to Fooden (2007), this 7-week period coincided with the beginning of the mating season which peaks “during the period November-January”.

Fourteen focal females from 2 out of the 3 groups, groups C and F, were subject to observation. These females had ages from 4 to 9 years old, more specifically 3 subadults (up to 4.5 years of age, the ones observed were old enough to already conceive) and 11 adults (5 years and older).  Seven of the focal females had the referred contraceptive implants and were from both groups. Among the contracepted females, there was 1 subadult and 6 adults, while among the non-contracepted females, there were 2 subadults and 5 adults. However, data collection happened blindly to the contraception stage of the focal females, as this information was only revealed after the on-site period of the study. These focal females are presented in table 1, which also shows which females had an infant to nurse during the period of data collection.

**Table 1: Focal females' description and respective individual information**

| Female ID | Female Photo | Group | Year of birth | With baby born in 2022? | C vs NC |
|---|---|---|---|---|---|
| F59 |  | C | 2013 | No | NC |
| F62 |  | F | 2013 | No | C |
| F64 |  | F | 2013 | Yes | NC |
| G52 |  | C | 2014 | Yes | NC |

| | | | | | |
|---|---|---|---|---|---|
| G54 |  | C | 2014 | No | C |
| G58 |  | C | 2014 | No | C |
| H51 |  | F | 2015 | No | C |
| H53 |  | C | 2015 | No | C |
| J51 |  | F | 2017 | Yes | NC |

| | | | | | |
|---|---|---|---|---|---|
| J52 |  | F | 2017 | No | C |
| J55 |  | C | 2017 | Yes | NC |
| K54 |  | C | 2018 | Yes | NC |
| K62 |  | F | 2018 | No | NC |
| K64 |  | F | 2018 | Yes | C |

Legend: ND – No Data; C – Contracepted; NC – Non-contracepted.

Each focal female was observed in protocols of thirty minutes, at least 1 morning (until 12:30 p.m.) and 1 afternoon (after 12:30 p.m.) per week. During the protocols, the interactions of relevance to register were inspections of the anogenital swelling by a group member (either male or female), mounts, and copulations, or attempts of these. Whenever an interaction was observed within the last 5 minutes of the protocol, it was prolonged 10 minutes as it shows that the female was socially active and could have a relevant interaction during this time. Protocol observations where females were out of view for over 5 minutes were noted as unfinished and discarded from the weekly observation counts, as within this unfollowed period the female could have had a relevant interaction that would not have been seen and, therefore, would not be accounted for. The order of the focal protocols each week was random and at different hours of the day for each female, always trying to spread timing and context as equally as possible per focal female. Focal females were not re-followed until all of them had the two weekly observations mentioned.

On-site, each time a protocol started, it was documented on personalized tables the date, starting and ending time of the observation, the number given to the protocol, and which female was being followed. Additionally, it was registered if the protocol was completed or if it had any interruption (and, if so, with a description of what happened – e.g.: the female disappeared to the top of a tree). Every time an interaction was observed, the said interaction was filmed (when possible) and, as soon as possible, the details of the interaction were registered on Google Sheets tables. Besides what was already pointed out, for each interaction, the following details were noticed:

- Inspector ID: The individual's ID that inspected the respective female.
- Who initiated: out of the female and the inspector, who initiated the interaction.
- Swelling dirt: If the focal female had dirt on the swelling and, if so, what, and how much dirt there was. This quantity was, whenever possible, noted as what it was (e.g. a leaf) and how much of the swelling it occupied (e.g. only one leaf on the right side of the swelling).
- Swelling semen: If the focal female had semen on the swelling and, if so, how much semen there was. This quantity was scored as in how much of the swelling it occupied (e.g. covering the left side of the swelling).
- Looking: If the inspector looked at the swelling during the interaction – when the inspector stared at the anogenital swelling at close range (≤1m). It was also noted if there was a particularity to the action, like if he just took a glance at the swelling, if he stared at it for a long time, or even if he turned around his way specifically to have a look at the swelling.
- Grabbing: If the inspector grabbed the swelling during the interaction – when the inspector held the anogenital swelling by the sides, specifying if he did so with one or both hands/only on one or both sides of the swelling.
- Smelling: If the inspector smelled the swelling during the interaction – when the inspector placed his nose at close range to the anogenital swelling (≤3 cm).

- Touching and smelling hand: If the inspector touched the swelling and smelled its hand after doing so during the interaction.
- Touching without smelling hand: If the inspector touched the swelling and did not smell its hand after doing so during the interaction.
- Copulation: If there was a just mount, copulation (mount with thrusting movements of the hips), or neither.
- Copulation calls: If there were copulation calls and, if so, when they happened (before, during, and/or after the copulation).
- Other observations: If there was anything else relevant to note, such as what happened before and/or after the interaction (e.g. aggression, grooming), the place it occurred on (e.g. on top of a tree, during feeding shows, etc.)
- Recorded?: If the interaction was recorded.

Some interactions observed did not have an inspection before copulation or mount. Therefore, when saying "interaction" it includes both with and without inspection, and when saying "inspection" it refers only to the act of inspection itself. The swelling dirt and semen were noted to understand if these possible swelling alterations would affect the use of olfactory cues by the inspectors.

Sexual interactions observed outside the study protocols involving focal and non-focal females from the two observation groups were also registered but as *ad libitum* data. These *ad libitum* observations were documented with the same method and variables as previously described, only, instead of starting and ending times, it was noted the daytime during which they occurred (morning or afternoon), and no protocol number was attributed.

Whenever possible, these interactions were recorded by one observer (the author) on a digital video camera (Panasonic HC-V180) or an iPhone 13. In every protocol, a photo of the respective focal female anogenital swelling was taken on an iPhone 13 to assess the swelling changes during the period of observations (details of these analyzes in described in more detail in section "4.2.3. Females Reproductive State"). Off-site, all these videos were carefully analyzed, and the data were reviewed and organized into final excel tables.

At the end of the study, there was a total of 294 swelling photos and 170 videos, of which 87 were protocol videos, and 83 were *ad libitum* data (table 2). As for the *ad libitum* data, 275 observations were made (171 in the morning period and 104 in the afternoon) involving females from groups C and F, born between 2000 and 2018, both contracepted and non-contracepted. In the end, there was a total of 83 videos made of non-focal observations.

**Table 2: Study focal observations per female**

| Female | Group | Total duration of observations (h:m:s) | | Total of photos | Total of videos |
|---|---|---|---|---|---|
| | | Morning | Afternoon | | |
| F59 | C | 05:30:00 | 05:04:15 | 21 | 3 |
| F62 | F | 05:23:00 | 05:30:00 | 22 | 2 |
| F64 | F | 06:03:25 | 04:59:30 | 22 | 0 |
| G52 | C | 05:00:00 | 05:30:00 | 21 | 0 |
| G54 | C | 04:54:00 | 06:09:55 | 22 | 26 |
| G58 | C | 05:02:10 | 05:45:45 | 21 | 21 |
| H51 | F | 05:18:00 | 06:13:30 | 22 | 30 |
| H53 | C | 05:08:10 | 05:00:30 | 21 | 1 |
| J51 | F | 05:40:00 | 04:47:00 | 21 | 1 |
| J52 | F | 05:33:45 | 04:40:30 | 21 | 0 |
| J55 | C | 04:59:56 | 05:06:40 | 20 | 2 |
| K54 | C | 05:00:00 | 05:00:00 | 20 | 0 |
| K62 | F | 05:00:00 | 05:00:00 | 20 | 1 |
| K64 | F | 05:30:00 | 04:30:00 | 20 | 0 |
| Total | C, F | 74:02:26 | 73:17:35 | 294 | 87 |

Legend: C – Contracepted; NC – Non-contracepted

### 4.2.3. Females Reproductive State

The swelling data collected, more specifically the anogenital swelling photos taken, were used to determine the reproductive cycle phase of the focal females since the swelling size increases as ovulation approaches (Möhle et al. 2005). For that, the program GIMP 2.10.32 (revision 1) was used to measure the distance between the lateral borders of the circumanal swellings and the distance between the lateral edges of the ischial callosities, thereby calculating the swelling size in relation to the callosities' size over the time of observation. Figure 4, adapted from a figure from a research article of Möhle et al. (2005), is a schematic picture for better understanding of the measures taken. As individual beings, the females show different sizes of their bodies and, more specially, their swellings. Thus, in order to compare the swelling changes between females, it was needed to resort to relative size measurements rather than absolute values to normalize the swelling sizes and have a better perspective of the changing patterns. This, in addition to an observational assessment of each photo, contributed to classifying the relative size of the anogenital swellings using a scale from 1 to 3 described by Young et al (2013) and presented in table 3.

Furthermore, the photos were also analyzed to determine the angle at which the swellings were captured. This measurement was taken by placing a protractor in front of the computer screen with each photo opened. The photos with angles bigger than 40º were removed from the analysis as they were less likely to show reliable size results. Table 4 shows the focal females' smallest and largest swelling photos (excluding the ones with bigger photo angles) with the respective dates the photos were taken, as well as the respective swelling sizes measured.

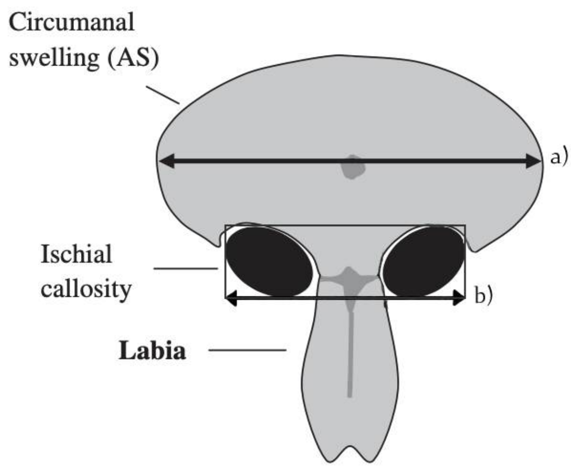


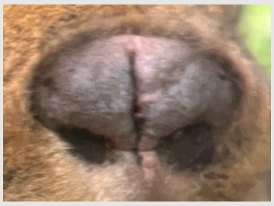
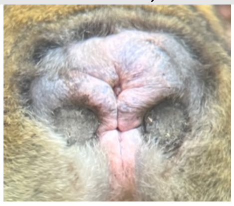
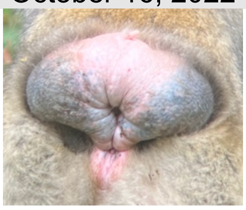
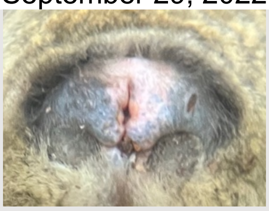
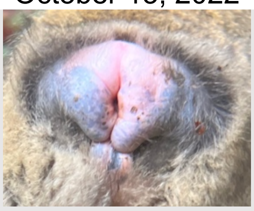

**Figure 4: Schematic picture of the two measures used to classify the anogenital swellings (adapted from Möhle et al. 2005)**

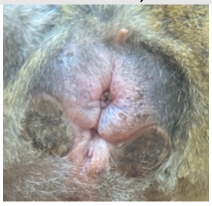
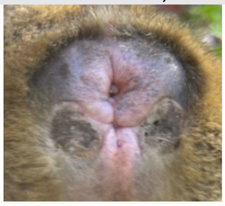
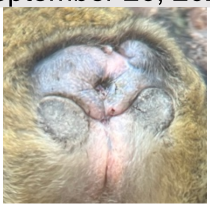
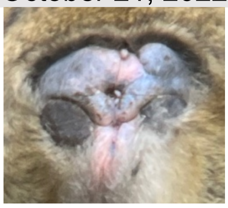
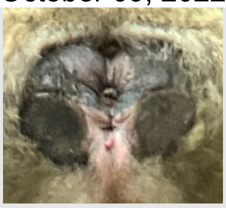
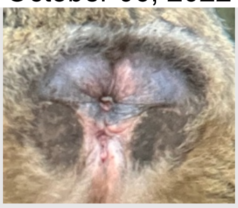
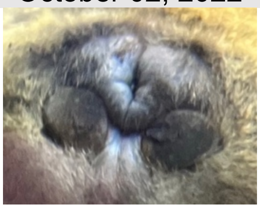
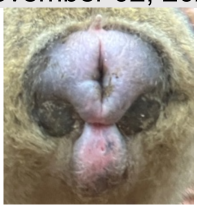
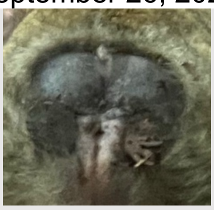
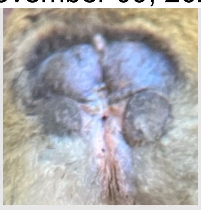
Legend: a) width of the circumanal swelling; b) width of the callosities

**Table 3: "Classification of fertile and swelling periods" by Young et al. 2013**

| Grade | Description |
|---|---|
| 1 | "a female with no or minimal swelling or minimal size" (Young et al. 2013) |
| 2 | "a partial swelling was present" (Young et al. 2013) |
| 3 | "a maximum swelling was present (tumescence, i.e. the swelling was at maximum size with no wrinkles, and there was protrusion of all genital structures, and this was the period of maximum turgidity)." (Young et al. 2013) |

**Table 4: Focal females' minimum and maximum swelling photos taken with respective dates and swelling sizes measured**

| Female ID | Group | C vs NC | Minimum swelling photo and date | Maximum swelling photo and date | Minimum Swelling Size | Maximum Swelling Size |
|---|---|---|---|---|---|---|
| F59 | C | NC |  September 25, 2022 |  November 11, 2022 | 0.8757 | 1.2249 |
| F62 | F | C |  October 04, 2022 |  October 02, 2022 | 1.1851 | 1.3285 |
| F64 | F | NC |  September 26, 2022 |  October 12, 2022 | 1.1478 | 1.3163 |
| G52 | C | NC |  September 25, 2022 |  October 16, 2022 | 0.9233 | 1.0891 |
| G54 | C | C |  September 29, 2022 |  October 13, 2022 | 1.4331 | 2.1993 |
| G58 | C | C |  October 13, 2022 |  November 01, 2022 | 0.9038 | 1.3677 |
| H51 | F | C |  October 04, 2022 |  November 10, 2022 | 1.2939 | 1.4885 |

| | | | | | | |
|---|---|---|---|---|---|---|
| H53 | C | C | <br>October 05, 2022 | <br>November 07, 2022 | 0.6024 | 1.0280 |
| J51 | F | NC | <br>October 30, 2022 | <br>November 06, 2022 | 0.9330 | 1.1084 |
| J52 | F | C | <br>September 26, 2022 | <br>October 21, 2022 | 0.8452 | 1.1227 |
| J55 | C | NC | <br>October 09, 2022 | <br>October 06, 2022 | 0.8519 | 0.9888 |
| K54 | C | NC | <br>October 02, 2022 | <br>November 02, 2022 | 0.7440 | 0.9643 |
| K62 | F | NC | <br>September 26, 2022 | <br>November 09, 2022 | 0.6270 | 1.0347 |
| K64 | F | C | <br>September 26, 2022 | <br>October 25, 2022 | 0.7715 | 1.0273 |

Legend: C – Contracepted; NC – Non-contracepted.

### 4.2.4. Statistical Analysis

Statistical Analysis was conducted in R version 4.2.1 (R Core Team, Vienna, Austria, 2022). Two groups of analyses (A. Contraception impact analysis and B. Minimum swelling size correlation study) were conducted to assess if there was a significant difference between the variables compared (the significance level considered for all statistical tests was p-value<0.5 as the confidence interval computed was 95%). The data used for the analysis were the sum of both protocol and *ad libitum* observations and, whenever time was needed, the durations used were the protocol observations' durations.

The contraception impact analysis (A) aimed to compare the data per female to assess if there was a significant difference in the parameters tested and explained below. These parameters included the different trends between females with and without contraception, the effect of the presence or not of swelling dirt, and the frequency of sniffs during inspections. The final plan for group 'A. Contraception impact analysis' with the variables explored and the respective statistical tests used are summarized in table 5.

In this first set of tests, the starting point was to analyze the number of inspections observed per focal female (Analysis A.I of tables 5 and 8). For this first analysis, a Mann-Whitney U test was used with the predictor variable being whether the females had or did not have contraceptive and the response variable being the calculated number of inspections per hour observed during the focal protocols. Then, the focus was to assess the percentage of inspections with sniffs (Analysis A.II of tables 5 and 8) using again a Mann-Whitney U test. Here the predictor variable stayed the same as before (females with and without contraception) and the response variable was the calculated rate of inspections with sniffing cues. The percentage of inspections leading to copulation (Analysis A.III of tables 5 and 8) was tested, again with a Mann-Whitney U test. The predictor variable remained the contraceptive state of the females and the response variable was the calculated rate of inspections leading to copulation observed. Another Mann-Whitney U test was used to analyze the trend in change in swelling size between the contracepted and non-contracepted females (Analysis A.IV of tables 5 and 8). Here the predictor variable was still whether the females had contraceptive implants or did not have them, and the response variable was a calculated value of the maximum swelling size divided by the minimum swelling size observed – hence the significance of the calculated anogenital swelling sizes from the photos taken be in relative values, as mentioned in section '4.2.3. Females Reproductive State'. To try to determine the timing (when possible) that the females started to exhibit an increase in the anogenital swelling size (Analysis A.V of tables 5 and 8), graphs were made by converting the dates of the observations from the Gregorian calendar to Julian Dates (JD). The following analysis was about evaluating the difference in the percentage of inspections between when females had dirt on their swelling and when they did not (Analysis A.VI of tables 5 and 8). To do this a Wilcoxon signed rank test with continuity correlation was used, comparing 2 levels of a predictor variable: inspections with or without dirt. Following, to examine the percentage of inspections with sniffs at swelling sizes 1/2/3 (Analysis A.VII of tables 5 and 8) it was needed to check the classifications given to the swelling sizes explained in the previous section '4.2.3. Females Reproductive State'. For this, it was computed the rate of inspections with sniffs per female at the 3 swelling size grades. However, it was noted that only 3 females (F59, H53, and K62) showed swelling grades 1 and 3 and, therefore, it was not meaningful to compare 1:3. Thus, Wilcoxon tests were run to compare swelling sizes 1:2 and 2:3. Lastly in this group of tests, analysis A.VIII of tables 5 and 8 aimed to assess the percentage of copulations with copulation calls between females with and without contraception. For this, the Mann-Whitney U test was once again used. The predictor variable was once more whether the females had or did not have contraceptive, and the response variable was the newly calculated variable rate of copulations with copulation calls observed during the focal protocols. In the initial plan, another parameter was of interest to investigate: the trend (if there was one) of the percentage of inspections with sniffs leading to copulation. For this, an attempt was made to calculate the rate of inspections with sniffing behavior that preceded copulations. However, this value could not be calculated as copulations after inspections with sniffs were not registered (as exposed in section '4.3. Results'). Thus, no test could be run for this question.

The minimum swelling size correlation study (B) was about correlating the minimum swelling size per female with the parameters tested and explained below – the minimum swelling size was used as a standpoint for the general size of the females' swelling. The final plan for group 'B. Minimum swelling size correlation study' with the variables explored and the respective statistical tests used are summarized in table 6.

In this second set of tests, given that the minimum swelling size per female was a continuous variable, correlation tests were run: Spearman's rank correlation test and Pearson's product-moment correlation test. The Spearman's rank correlation test was chosen for analyses involving the number of inspections per hour, the percentage of inspections with sniffs, and the percentage of inspections leading to copulations. This choice is due to the non-parametric nature of the data, potential ordinal characteristics, robustness to outliers, and the expectation of monotonic but not necessarily linear relationships. The first parameter investigated was how the minimum swelling size that the females showed influenced the number of inspections per hour (Analysis B.I of tables 6 and 9). The chosen test was a Spearman's rank correlation test using the variables 'inspections per hour' (calculated) and 'minimum swelling size'. Secondly, analysis B.II of tables 6 and 9 assessed about the percentage of inspections with sniffs. A Spearman's rank correlation test was once again used with the variables being the rate of inspections with sniffs and the females minimum swelling size registered. One last Spearman's rank correlation test was used for the percentage of inspections leading to copulations (Analysis B.III of tables 6 and 9). In this test, the variables were the calculated rate of inspections followed by copulations and again the minimum swelling size showed by the females. The last test was to investigate the timing of the swellings (Analysis B.IV of tables 6 and 9), more specifically to find if there was a trend between the minimal swelling size of the females and the timing it started to increase. This analysis required to see the results of the test 'A.V. Onset of swelling increase' and investigate this parameter only using the females for which it was possible to determine the date their swelling started to increase. Thus, a Pearson's product-moment correlation test was run using as variables the minimum swelling size and Julian day of the onset swelling increase of the females selected as described before. This test is appropriate because both variables are continuous, and there is a linear relationship and normal distribution. In the initial plan, there was also another parameter of interest: the percentage of inspections with sniffs leading to copulation. For this, an attempt was made to calculate the rate of inspections with sniffing behavior that preceded copulations. However, and once again, this value could not be calculated as copulations after inspections with sniffs were not registered (as exposed in section '4.3. Results'). Therefore, no test could be run to assess this parameter.

**Table 5: A. Contraception impact analysis plan and respective analysis**

| Analysis Plan | | Analysis |
|---|---|---|
| I. | Number of inspections | Mann-Whitney U test |
| II. | Percentage of inspections with sniffs | Mann-Whitney U test |
| III. | Percentage of inspections leading to copulation | Mann-Whitney U test |
| IV. | Change in swelling size | Mann-Whitney U test |
| V. | Onset of swelling increase | Graphs made for each female are present below in section '4.3.Results', figures 5, 6, and 7 |
| VI. | Percentage of inspections when dirt vs no dirt on the swelling (paired per female) | Wilcoxon signed rank test with continuity correlation |
| VII. | Percentage of inspections with sniffs at swelling sizes 1/2/3 (paired per female) | Wilcoxon tests between swelling sizes 1 and 2, and between swelling sizes 2 and 3. There were not significant amount of data to compare sizes 1 and 3. |
| VIII. | Percentage of copulations with copulation calls | Mann-Whitney U test |

**Table 6: B. Minimum swelling size correlation study's plan and respective analysis**

| Analysis Plan | | Analysis |
|---|---|---|
| I. | Number of inspections per hour. | Spearman's rank correlation test |
| II. | Percentage of inspections with sniffs | Spearman's rank correlation test |
| III. | Percentage of inspections leading to copulation | Spearman's rank correlation test |
| IV. | Timing of swellings | Pearson's product-moment correlation test |

### 4.3. Results

At the end of the study, there were a total of 147.3 hours of protocol observations. During both protocol and *ad libitum* observations made by the observer, involving the focal females, 194 interactions were observed: 172 of these 194 had inspections associated, and 22 of the same 194 were copulations or mounts that did not have a prior inspection. Concerning the individual who started the interactions, 133 (out of the 194) were initiated by males, and 41 (out of the 194) were initiated by females.

In the total of 172 inspections mentioned, 62 (out of the 172) inspections were followed by copulation, while 109 (out of the 172) did not lead to further sexual interaction. Furthermore, 25 (out of the 172) inspections included an olfactory interaction, while 147 (out of the 172) did not. The total of inspections when females had dirt on the anogenital swelling was 59 (out of the previous 172), 108 (out of the previous 172) when there was no dirt on the swelling.

When paying special attention to the 25 inspections with olfactory behavior, 13 (out of the 25) inspections with sniffs were initiated by males, and 12 (out of the 25) inspections with sniffs initiated by females. Moreover, it was possible to register that 2 (out of the 25) happened when the focal individual had dirt on the swelling and 21 (out of the 25) when there was not. During the 7 weeks of observation, copulations after sniffing behavior were not seen (0 out of the 25).

Additionally, it was registered a total of 109 mounts and copulations: 18 (out of the 109) were mounts, and 91 (out of the 109) were copulations. Out of these 91 copulations, 37 have copulation calls associated, and 54 did not.

These results are summarized in table 7 for easier visual access.

**Table 7: Summary of the results of the observations made on-site**

| Observation | Total |
|---|---|
| Duration of focal protocols | 147.3 h |
| Total of interactions | 194 |
| Interactions initiated by males | 133 of 194 |
| Interactions initiated by females | 41 of 194 |
| Total of mounts and copulations without previous inspection | 22 of 194 |
| Total of inspections | 172 of 194 |
| Inspections followed by copulation | 62 of 172 |
| Inspections not followed by copulation | 109 of 172 |
| Inspections when there was dirt on the focal female anogenital swelling | 59 of 172 |
| Inspections when there was no dirt on the focal female anogenital swelling | 108 of 172 |
| Inspections with sniffs | 25 of 172 |
| Inspections without sniffs | 147 of 172 |
| Inspections with sniffs initiated by males | 13 of 25 |
| Inspections with sniffs initiated by females | 12 of 25 |
| Inspections with sniffs when there was dirt on the focal female anogenital swelling | 2 of 25 |
| Inspections with sniffs when there was no dirt on the focal female anogenital swelling | 21 of 25 |
| Inspections with sniffs followed by copulation | 0 of 25 |
| Inspections with sniffs not followed by copulation | 25 of 25 |
| Total of mounts and copulations | 109 |
| Total of mounts | 18 of 109 |
| Total of copulations | 91 of 109 |
| Copulations with copulation calls | 37 of 91 |
| Copulations without copulation calls | 54 of 91 |

As far as the R analysis goes, in the ‘Contraception impact analysis’ (A) only one test turned out to show a significant difference between the variables in the case - ‘A.VI. Percentage of inspections with sniffs when dirt vs no dirt on the swelling (paired per female)’ from tables 5 and 8, explained further down. Table 8 summarizes the contraception impact analysis test questions and their conclusions.

Firstly, it was found that non-contracepted females did not show more quantity of inspections than contracepted females (MWU: N=14, U=34, p=0.248 – analysis ‘A.I. Number of inspections’ of tables 5 and 8). Secondly, non-contracepted females did not display a higher percentage of inspections with sniffs than contracepted females (MWU: N=14, U=24, p=0.375 – analysis ‘A.II. Percentage of inspections with sniffs’ of tables 5 and 8). The third test (‘A.III.

Percentage of inspections leading to copulation' of tables 5 and 8) concluded that females without contraceptive implant did not reveal a higher rate of inspections followed by copulation compared to contracepted females (MWU: N=14, U=17, p=0.935). As far as changes in swelling size go, non-contracepted females did not exhibit a bigger change in swelling size during the period of observations compared to females with the Implanon NXT contraceptives (MWU: N=14, U=32, p=0.383 – analysis 'A.IV. Change in swelling size' of tables 5 and 8). For the assessment of the onset of swelling increase (analysis 'A.V. Onset of swelling increase' of tables 5 and 8), graphs (presented below in figures 5, 6, and 7) were made for each female. It was possible to determine the dates of the start of the increase of the swelling for the following females: F59 (Julian day 289 / October 16, 2022), G58 (Julian day 283 / October 10, 2022), H51 (Julian day 279 / October 06, 2022), H53 (Julian day 291 / October 18, 2022), J52 (Julian day 290 / October 17, 2022), and K62 (Julian day 286 / October 13, 2022). As for analysis, in tables 5 and 8, 'A.VI. Percentage of inspections with sniffs when dirt vs no dirt on the swelling (paired per female)', revealed that there were more inspections with sniffing cues when there was no dirt on the anogenital swelling than when there was (Wilcoxon signed-rank test: N=14, V=10, p=0.045) – this result is clear on the boxplot presented below in graph 1. To examine the percentage of inspections with sniffs at swelling sizes 1/2/3 (analysis A.VII of tables 5 and 8) it was needed to run 2 separate tests to compare swelling sizes 1:2 and 2:3. The results showed that swelling size grade 2 did not have a higher rate of inspections with sniffs than grade 1 (MWU: N=5, U=3, p=0.584), and that swelling size 3 also did not show a higher percentage of inspections with sniffing cues than grade 2 (MWU: N=5, U=11, p=0.438). Lastly, the analysis 'A.VIII. Percentage of copulations with copulation calls' (of tables 5 and 8) did not show a higher rate in females without contraception compared to females with contraception (MWU: N=14, U=15, p=0.227).

**Table 8: A. Contraception impact analysis – tests questions and respective results and conclusions**

| | Tests questions | Results | Conclusions |
|---|---|---|---|
| I. | Number of inspections | MWU: N=14, U=34, p=0.248 | Non-contracepted females did not show more quantity of inspections than contracepted females. |
| II. | Percentage of inspections with sniffs | MWU: N=14, U=24, p=0.375 | Non-contracepted females did not display a higher percentage of inspections with sniffs than contracepted females. |
| III. | Percentage of inspections leading to copulation | MWU: N=14, U=17, p=0.935 | Non-contracepted females did not reveal a higher rate of inspections |

| | | | |
|---|---|---|---|
| | | | followed by copulation compared to contracepted females. |
| IV. | Change in swelling size | MWU: N=14, U=32, p=0.383 | Non-contracepted females did not exhibit a bigger change in swelling size during the period of observations compared to contracepted females. |
| V. | Onset of swelling increase | NA | Graphs made for each female are presented below in figures 5, 6, and 7. It was possible to determine the onset of swelling increase date for 6 females: F59, G58, H51, H53, J52, and K62. |
| VI. | Percentage of inspections with sniffs when dirt vs no dirt on the swelling (paired per female) | Wilcoxon signed-rank test: N=14, V=10, p=0.045 | There were more inspections with sniffs when there was no dirt on the swelling. |
| VII. | Percentage of inspections with sniffs at swelling sizes 1/2/3 (paired per female) | MWU 1/2: N=5, U=3, p=0.584; MWU 2/3: N=5, U=11, p=0.438 | Swelling size grade 2 did not have a higher rate of inspections with sniffs than grade 1, and swelling size grade 3 also did not show a higher percentage of inspections with sniffs than grade 2. |
| VIII. | Percentage of copulations with copulation calls | MWU: N=14, U=15, p=0.227 | Non-contracepted females did not show a higher rate of copulations with copulation calls compared to contracepted females. |

Legend: NA – Not Applied; MWU - Mann-Whitney U test; N - sample size; U - set of data; p - p-value


**Figure 5: Analysis Plan Group A.VI. results of females F59, F62, F64, G52, G54, and G58, each graph with its respective female indicated**


**Figure 6: Analysis Plan Group A.VI. results of females H51, H53, J51, J52, J55, and K54, each graph with its respective female indicated**

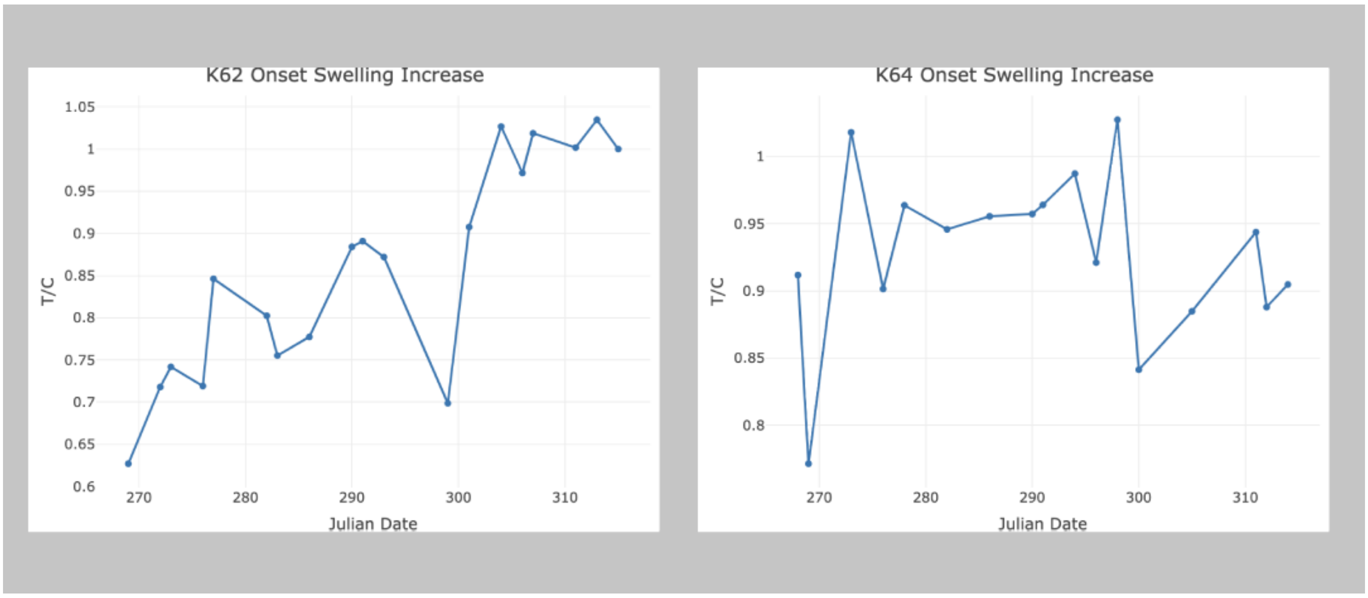
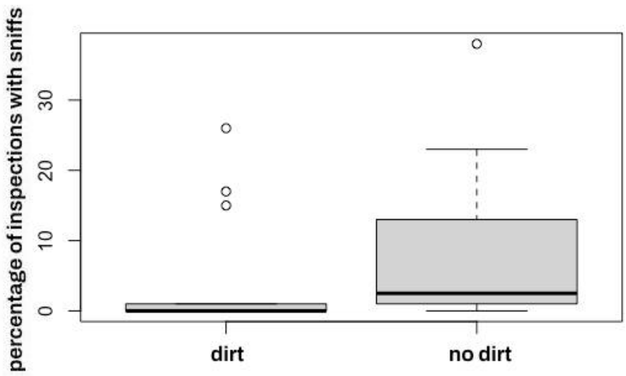



**Figure 7: Analysis Plan Group A.VI. results of females K62, and K64, each graph with its respective female indicated**

**Graph 1: Boxplot result of test A.VI. Percentage of inspections with sniffs when dirt vs no dirt on the swelling**




Legend: dirt – the percentage of inspections when there was dirt on the swelling, no dirt – the percentage of inspections when there was no dirt on the swelling

In the 'B. Swelling size correlation study', only two tests turned out with significant results. Table 9 summarizes the 'B. Swelling size correlation study' test questions and their conclusions.

Test 'B.I. Number of inspections per hour' (of tables 6 and 9) showed that the minimum swelling sizes did not influence the frequency of inspections (Spearman: N=14, S=349.54, p=0.425). The second test run of this group plan concluded that females with larger minimum anogenital swellings were sniffed more (Spearman: N=14, S=258.51, p=0.038, $\rho$=-0.603 - analysis 'B.II. Percentage of inspections' of tables 6 and 9). The third test conducted on this second set of tests indicated that females without contraception did not show a higher rate of inspections followed by copulation than contracepted females (Spearman: N=14, S=227.87, p=0.526, ρ=0.203 - analysis 'B.III. Percentage of inspections leading to copulation' of tables 6 and 9). The last test of the analysis was computed for the 6 females who showed a clear date of onset swelling increase in the previous test 'A.V. Onset of swelling increase': F59, G58, H51, H53, J52, and K62. This test revealed that the focal females with smaller minimum swelling started to increase in swelling size later (Pearson, N=6, t=-3.119, p=0.036, *r* = -0.842 - analysis 'B.IV. Timing of swellings' of tables 6 and 9).

**Table 9: B. Swelling size correlation study – tests questions and respective results and conclusions**

| Tests questions | Results | Conclusions |
|---|---|---|
| I. Number of inspections per hour | Spearman: N=14, S=349.54, p=0.425 | The minimum swelling sizes did not influence the frequency of inspections. |
| II. Percentage of inspections with sniffs | Spearman: N=14, S=258.51, p=0.038, ρ= -0.603 | As the minimum swelling increases, the rate of inspections with sniffs decreases. |
| III. Percentage of inspections leading to copulation | Spearman: N=14, S=227.87, p=0.526, ρ=0.203 | Non-contracepted females did not show a higher rate of inspections followed by copulation than contracepted females. |
| IV. Timing of swellings | Computed for the females F59, G58, H51, H53, J52, and K62: Pearson, N=6, t= -3.119, p=0.036, *r* = -0.842 | The females that start to increase in swelling size later are the ones with the smaller minimum swelling. |

Legend: NA – Not Applied; N - sample size; S - Spearman's rank correlation statistic; t - Pearson's correlation statistics; p - p-value; ρ - Spearman's rank correlation coefficient; *r* - Pearson's rank correlation coefficient

## 4.4. Discussion

The primary focus of observation was to assess how Barbary macaques inspected swellings depending on various predictors (such as the available visual cues and olfactory assessment), and if these available cues affected subsequent sexual behavior. Regarding the sense of smell, the main sense of interest of this research, only 25 of the 172 (approximately 14.5%) anogenital inspections observed included olfactory behavior.

This study aimed to assess if males would rely on olfactory cues to gain information on the females' reproductive phase and, thereby, be able to differentiate the individuals with the Implanon NXT contraceptive implants. However, the details recorded of the interactions observed and the tests run during the analysis focused on much more than just understanding the different approaches by the males towards contracepted and non-contracepted females. The swelling information collected was important to determine the reproductive cycle phase at which the females were in each interaction, as well as grasping how the contraceptive would affect their evidence of external signs of fertility. Moreover, recording and analyzing the other senses utilized by males while exploring the females' anogenital swellings in their encounters helped understand the significance attributed to olfaction as well as the frequency with which they relied on it.

The expectation was that males would invest in mating with fertile females instead of spending their time and energy on females with contraception with which, therefore, they would not have a successful mating interaction. However, no evidence was found that male sexual behavior differs between contracepted and non-contracepted females.

Analysis of the collected data revealed no significant differences between females with contraceptives and females without in what comes to the changes in the anogenital swelling size. This appears to reflect in not many differences shown in the available cues to males to differentiate the females' contraceptive condition. These findings agree with the personal observations made by Simon et al. in their research at Affenberg Salem in 2020. In this investigation, they observed that both contracepted and non-contracepted females show similar anogenital swelling sizes as well as patterns of tumescence and detumescence. Such consideration points to accordance with the results obtained from the records of males' behavior towards both contracepted and non-contracepted females: no significant difference in terms of quantity of inspections, rate of sniffing inspections, nor the rate of inspections leading to copulation. Regarding the females' behavior, following the previous assumptions and results, it makes sense that the rate of copulations with copulation calls attained did not show significant values between contracepted and non-contracepted individuals.

In alignment with the outcomes discussed in the previous paragraph is a study by Young et al. (2013). In 2013, this researcher suggested that male Barbary macaques could not distinguish females on fertile and non-fertile reproductive cycles when they showed maximum swelling stages. No evidence was found that the contraceptive interferes with the development of the swelling sizes since results show increases of this parameter in contracepted females. Therefore, it makes sense that males would show similar behavior to females with equivalent anogenital swelling size to the moment of ovulation, regardless of whether one has contraception or, when not on contraception, is currently ovulating or not. This suggests that the Implanon NXT implant does not stop the changes in the size of the anogenital swelling. Thus, males will copulate when the female has a swelling the size that, according to previous articles, might correspond to the moment when the ovulation is supposed to occur, whether it happens or not. Despite that, studies on other species show evidence of males capable of distinguish contracepted from non-contracepted females. This is the case in a study that took place in the Duke Lemur Center in Durham, United States of America, by Crawford, Boulet, and Drea. This study focused on examining the effects of medroxyprogesterone acetate (MPA) contraceptive on olfactory communication during the mate choice of ring-tailed lemurs (*Lemur catta*). The subjects of this investigation included males and females who did and did not receive the said contraceptive injection. The authors concluded that the contraceptive changed the females' olfactory profiles and males could detect the difference in odor cues between contracepted and intact females (Crawford et al. 2011).

Nonetheless, it was of interest to register further external conditions, such as dirt on the females' anogenital swelling, to assess how would males manage the sexual inspection with such elements accessible to other senses, such as vision. A bigger frequency of inspections using olfaction was observed when the females did not have dirt on their swellings. This finding could be linked to the sequential strategy employed by olive baboons involving the utilization of multiple female sensory signals and cues to mitigate the costs of mate choice. By sequentially employing multiple cues, such as initial cues related to visual morphological traits like sexual swelling, males can identify females worthy of closer inspection for subsequent cues, such as olfactory and tactile cues, which are more costly to evaluate but provide more precise information about mate quality. This strategic approach has the potential to decrease the number of females requiring closer examination and, consequently, reduce the overall costs associated with mate choice (Rigaill et al. 2013). Therefore, males could visually recognize 2 parameters observed: the swelling size and dirt on the swelling. As said before, the swelling size is directly related to the timing of probable ovulation. As for the dirt on the swelling, it would be logical to think that the dirt might cover off some of the olfactory cues, or that, given the importance of grooming in this species, its visual detection could be associated with possible parasites that would affect the health of the male if he were to inspect. However, these are merely speculations as no studies were found on what non-human primates might relate to dirt on the female's swelling.

No copulations were registered after inspections with sniffs, a result also registered in research referenced before by Simon et al. (2022) that also took place at Affenberg Salem. There is a possibility that the fertile females might release olfactory cues and/or signals detectable from a great distance, so the males would not need to actively sniff the swellings to detect such fertility parameter. The study on olive baboons already mentioned agrees with this speculation. In this study's discussion, it is suggested that during the fertile phase, the strength of the olfactory cues is higher. Thus, males do not need to inspect the females directly to detect them (Rigaill et al. 2013).

Findings also indicate that the sniffing inspection rate decreased as the females' minimal swelling increased. The assumptions for the first 2 results previously presented might support this as well. The bigger absolute size of an anogenital swelling in Barbary macaque females may be detected by the males through visual assessment and thus be directly associated with the fertile phase, without the need to resort to olfactory cues. On another hand, and as presented before as well, the olfactory cues emitted could be intense enough that they could be detected at a larger range and so the males would not need to do a close sniffing inspection. In this case, a potential olfactory component cannot be excluded from what was recorded as only a visual inspection.

Furthermore, data revealed that females who started to increase swelling size earlier showed bigger minimal swelling size. This result might be related to the age of the females, their sexual activity history as well as their contraception backgrounds. It is logical to think that older females have more mature reproductive systems and, therefore, have a more developed anogenital swelling and faster development of the same, starting to mate earlier in the season as proposed by Kuester and Paul (1984). Moreover, the same researchers have also detected connections between the extent of the swellings and the females' reproductive background. When comparing swellings of the same individuals across successive breeding seasons, they noted that swellings tended to be smaller following the rearing of an infant in the previous season compared to a year of sterility, irrespective of whether the females conceived during that period (Küster & Paul 1984). Therefore, the development of the swellings could be a reflection of the females' contraceptive history, as individuals with contraception placed in previous breeding seasons would not have offspring to take care of and so could show larger anogenital swellings.

To confirm these last speculations concerning the growth of the anogenital swelling size, it is necessary to do a more in-depth investigation of each individual's background records. By analyzing the reproductive history in more detail, alongside their swellings and hormonal concentration, more conclusive results could be taken to clarify this finding. Additionally, to better understand the results presented, moreover confirm the conjectures made, additional test analysis, a longer period of observation, and observation during the peak of the mating season could make the difference. Extending the observation timeline would enable us to collect data during the most active period of mating interactions, while also monitoring the development of females throughout the reproductive season. This would include tracking the progression of swelling, observing their acceptance and interaction with males, and conducting a detailed analysis of their hormonal concentration levels. Therefore, this would be an important factor to clarify and strengthen the results obtained in the present research.

### 4.5. Study Limitations

The present research is not without limitations. The primary drawback was the time frame available for observations due to Affenberg Salem's availability to receive this project. In light of safety precautions for the trainee, the observations had to finish before the peak of the mating season. As explained before, an extended period of observation coinciding with the onset of mating interactions, the timeline of observations in this study, forward to the peak and cessation of the mating season, would be beneficial to collect more data and clarify some results obtained. The restricted useful time window has also limited the understanding of the females' anogenital swellings development. It would have been beneficial to observe the females for at least 1 entire reproductive cycle to be sure what the minimum and maximum swelling size for a given female was.

The sample size is a factor to think about. The number of focal individuals observed was large enough to draw statistically significant conclusions and consistent with previous studies. However, a bigger sample group is always preferable in scientific studies, as the bigger the sample the more representative of the population it is. Focal observations of more individuals would have given more reliable data.

The trainee had a period before data collection to learn how to walk among the individuals and collect data with minimal interference. However, human interference must be a factor to consider as, as minimal as it might have been, it cannot be completely eliminated. Furthermore, the semi-free enclosure that the studied population lives in has human interference from the visitors, an interference that is absent in some Barbary macaques' populations within their natural habitat. The degree of influence of these different living settings is yet to be understood.

An aspect that cannot be forgotten is the contraceptive implants. During the period of observations, and within the focal females' sample, some of the Implanon NXT implants had to be replaced. To do this, the individuals had to be patiently caught using walk-in traps, and only by the end of October did all the females have their contraceptive check completed for the breeding season ahead. Therefore, during data collection, there were females counted as contracepted that needed an Implanon NXT replacement, and the change could only be made in the last weeks of observations (more specifically, F62 and G54). The contraceptive implant is usually replaced before the dose gets so low that they can conceive again, so this does not seem to be a very worrying topic as these females should have had a sufficiently high dose before and after the replacement.

It would be beneficial to conduct a more detailed study on the variations in odor production and release among individuals. To do this, one could use, for example, the method used in a research for the same purpose in olive baboons: a collection of daily vaginal odor samples using sterile cotton swabs and then conducting a laboratory analysis. In the laboratory, the aim was to find the volatile components present in the vaginal odor discharge by resorting to gas chromatography-mass spectrometry (GC-MS) and solid-phase microextraction (SPME). However, besides the costs of this investigation, it is also an invasive and stressful method for the animals as there is the need to rub the swabs around the wall of the female's vulva (Vaglio et al. 2021). Additionally to this analysis, it would be interesting to consider their age, reproductive activity and history, fertility stage, anogenital swelling size, behavior, and, if possible material available for blood analysis, monitor the blood hormonal changes of each individual. This investigation would help us better understand and confirm the possibility that larger swelling sizes and the fertile cycle are directly related to odor levels and their long-range detection.

Given that this was an observational research and that it was not possible to conduct interobserver reliability assessment in every interaction (only the ones recorded in video), one cannot dismiss the element of subjectivity inherent in the human observer. Although the observer considered the behaviors as described in '4.2.2. Behavior Observation' to standardize the actions as much as possible, there were moments when the activities were more discrete and needed a judgment call by the observer on what to consider. An example is the behavior noted as looking at the swelling: apart from the very clear cases of visual assessment ("staring"), the observer had to rethink when a male looked at the female swelling in movement or even when he only walked towards her behind before jump right up to mount or copulate. In these examples, vision was counted as being used, although other observers may not have done the same consideration. Olfaction-wise, sometimes there was also doubt if there was a discrete sniff or not – but for this, the observer was stricter and more focused on the description of the behavior. Another movement that left sometimes more or less uncertain was to distinguish between mount and copulation since, at times, the motion that sets apart the two behaviors (the hip movement during copulation which was absent in just mounts) was very subtle. Likewise, there was some difficulty in the assessment of the cycle states via swelling sizes given the single observer. Capturing good-angled photos of the swellings at the same time as paying attention to the individual's behavior was at times challenging to do in parallel. A partner observer would be useful in this way as well.

The limitations presented highlight the significance of careful planning and may assist in the enhancement of research conditions in future studies.

## 5. Conclusion

In light of the findings of the present research, it can be concluded that Barbary macaque males do in fact rely on olfaction during sexual interactions. However, given its low frequency of use compared to visual assessments, it seems that olfaction does not take as a dominant role as vision. Regarding contraception, it did not seem to affect the development of the females' anogenital swelling as both contracepted and non-contracepted individuals showed changes in this anatomic aspect of much relevance in sexual interactions.

The absence of significant difference between females with and without contraception in different variables related to the inspection is following referred studies. This evidence indicates that, despite preventing females from getting pregnant, the Implanon NXT contraceptive does not seem to affect the changes in swelling size. Whether there are signs of infertility that do not perturb the mating behavior, males cannot detect those cues, or they read them but mate nonetheless is not clear. What is notable is that, as long as females expose a progressed anogenital swelling, then males will engage in sexual activity with them.

Results also agree with the data of previous studies that inspections with olfactory cues were not followed by copulation. This suggests that Barbary macaque males resort to olfactory cues more at an early stage of the females' both reproductive cycle and swelling size development. Thus, visual assessments seem to have a bigger role before mating interactions around what it is supposed to be the fertile phase as it was present in every sexual inspection prior to mounts or copulations. According to the literature, tumescence swelling is characteristic of the approach to the ovulation moment, which seems to be visually recognized by the males.

In conclusion, while there are certain limitations to the study, it provides valuable insights into the role of olfactory cues in sexual inspections during the process of male mate selection in Barbary macaques. Further research can be built upon these findings to expand our understanding of primate behavior and mate selection, and improve the living conditions of captive populations. Future research would benefit from a longer observation period and a more detailed analysis of reproductive records and hormonal profiles.

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# 7. Appendices

## Appendix 1: Scientific poster presented at Behavior 2023 (August 14th-20th, Bielefeld, Germany)