Brief Report

Folia Primatol 2003;74:75–79
DOI: 10.1159/000070000

Received: August 2, 2002
Accepted after revision: September 26, 2002

Vaginal Fatty Acids Produced by Chimpanzees during Menstrual Cycles

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Key Words
Olfactory signal · Sexual attractant · Fatty acids · Periovulatory period · Pheromone · Chimpanzees

Introduction

It is suggested that an olfactory signal is a proximate factor in menstrual synchronisation and ovulation suppression, as well as being a sexual attractant [Jolly, 1967; Michael and Keverne, 1968, 1970; Wallis, 1985]. Adult female chimpanzees *(Pan troglodytes)* are in maximal swelling for 10–13 days of their 32- to 36-day sexual cycle, and ovulation occurs during the last 1 or 2 days of the maximal swelling period [Graham, 1981]. Although observers are unable to determine from physical appearance when ovulation starts, male chimpanzees are reported to change their behaviour between quiescent swelling and maximal swelling or early tumescence and periovulatory periods [Tutin, 1979; Hasegawa and Hiraiwa-Hasegawa, 1983; Takasaki, 1985; Matsumoto-Oda, 1999]. If males can determine the timing of ovulation, one cue may be an olfactory signal.

Fox [1982] identified six fatty acids from the vagina of chimpanzees. No significant change in levels, however, has been seen between the cycling and non-cycling periods. Females have a fixed rhythm of sex hormone production that is said to influence the grade of substance secretion and olfaction [Wallis, 1992]. Swelling of sexual skin is related to secretion of oestrogen in the follicle period, and diminution is related to a decrease of oestrogen and increase of progesterone [Graham, 1981]. Vaginal mucus and low-grade fatty acids responding to the swelling in captive chimpanzees were quantified during this study.

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0015–5713/03/0742–0075$19.50/0

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Table 1. Mean cycle length of each female during April–September, 1999

Individual[1]	Age years	Non-ovulatory period, days				Cycle length days
		flat	partial swelling	maximal swelling, days		
				early tumescence	periovulatory	
Pan	14	8.8	1.7	10.5	4.0	31.0
Popo	16	11.5	3.3	10.0	4.0	31.8
Chloé	18	14.0	3.7	8.5	4.0	31.7
Pendesa	22	15.0	4.3	8.0	4.0	33.5

[1]The four subjects were raised by humans just after birth because their mothers refused to rear them. They have grown up and had experience of meeting other individuals at the Institute since being 0 (Pan and Popo), 2 (Pendesa) and 4 (Chloé) years old. They are now in a community of 11 individuals. These chimpanzees spent from 9:30 until 16:30 each weekday in an open enclosure (700 m^2) in two groups with other chimpanzees, except when engaged in cognition and learning experiments.

All the subjects are so habituated to the staff that they even tolerate injections. The chimpanzees were trained to come when called by staff and stand with their back toward them. They even tolerated having a swab inserted into their vagina.

Methods

Subjects

The subjects were four captive adult female chimpanzees in the Primate Research Institute, Kyoto University (table 1). The detailed breeding environment in the Institute is described in Ochiai and Matsuzawa [1997]. During the study period, one subject, Popo, was grouped with 2 males and 2 females, and the others were with a male and 2 females.

A.M.O. recorded turgidity according to three classifications: flat, partial swelling and maximal swelling. There was no significant correlation between cycle length and age of the subjects from April to September in 1999 (table 1, Kendall rank correlation, $t = 0.19$, $p = 0.38$). We call the last four days of the maximal swelling period, in which the possibility of impregnation is high, the 'periovulatory period' according to Goodall's [1986] definition, and call the remainder of the maximal swelling period 'early tumescence'. We grouped the flat, partial swelling and early tumescence periods, in which females were not likely to conceive even if they copulated, into the 'non-ovulatory period'.

Sample Collection

Samples were collected 3 times/week from August 11 to September 17, 1999. One author prepared a swab consisting of cotton (1.3 × 2.0 cm) soaked with 1 ml of 30% ethanol solution and a stick (13.0 cm), while another prepared the chimpanzee. Part of the cotton swab was inserted into the vagina and a sample was collected. The swab was put in a 15-ml plastic centrifugal pipe and preserved at –20°C. We tried to ensure that the amount of ethanol on the cotton and the amount of sample collected was the same each time.

Chemical Analysis

Liquid was collected by centrifuging the samples at 1,000 *g* for 1 min at 4°C. Two microliters of the supernatant was put on a Shimazu Gas Chromatograph GC-9APTF with a Silar-10C 10% Chromosorb W column (80–100 mesh, 2.1 m × 3.2 mm, Supelco). The fraction was ionized and each detected peak was integrated by Shimazu Chromatopack C-R6A. The elution conditions were: column temperature 160–240°C (4°C increase/min), sample evaporation chamber temperature 260°C, detection temperature 260°C and N_2 gas flow rate 60 ml/min. Data were obtained as areas. Determination of each fatty acid was performed by comparisons with the position of the peaks of standard fatty acids. The percentage of each

Table 2. Mean amount of mucus and fatty acids in each period

Individual	Period							
	flat	samples	partial swelling	samples	early tumescence	samples	periovulatory	samples
Mucus or fatty acid								
Mucus[1]*, ml*								
Pan	1.14 (0.86–1.37)	7	0.88 (0.72–1.06)	4	1.25 (1.16–1.36)	4	0.98 (0.67–1.40)	2
Popo	0.90 (0.65–1.21)	7	1.02 (0.96–1.08)	2	1.17 (0.93–1.30)	6	1.98 (1.26–2.69)	2
Chloé	1.10 (0.76–1.96)	7	0.86 (0.74–1.05)	3	1.06 (0.94–1.20)	6	0.69	1
Pendesa	1.11 (0.67–1.40)	6	1.00 (0.87–1.24)	4	0.89 (0.79–0.99)	6	1.19	1
Acetic acid[2]*, %*								
Pan	0.70 (0.00–3.98)	7	0.65 (0.00–2.15)	4	0.00 (0.00–0.00)	4	0.00 (0.00–0.00)	2
Popo	0.48 (0.00–1.49)	7	1.07 (0.96–1.19)	2	1.15 (0.00–3.78)	6	0.81 (0.69–0.93)	2
Chloé	1.18 (0.00–3.61)	7	2.19 (0.83–4.38)	3	2.04 (0.80–4.66)	6	2.61	1
Pendesa	0.09 (0.00–0.29)	6	0.13 (0.00–0.51)	4	0.32 (0.00–1.14)	6	1.42	1
Propionic acid[2]*, %*								
Pan	0.11 (0.00–0.67)	7	0.29 (0.00–0.60)	4	0.04 (0.00–0.18)	4	0.00 (0.00–0.00)	2
Popo	0.14 (0.00–0.71)	6	0.32 (0.00–0.63)	2	0.24 (0.00–1.05)	5	0.08 (0.00–0.16)	2
Chloé	0.32 (0.00–0.60)	7	0.28 (0.00–0.62)	3	0.25 (0.00–0.63)	6	0.31	1
Pendesa	0.03 (0.00–0.11)	6	0.01 (0.00–0.05)	4	0.02 (0.00–0.09)	6	0.11	1
Butyric acid[2]*, %*								
Pan	96.28 (86.71–99.19)	7	96.23 (92.75–98.46)	4	97.23 (95.17–98.49)	4	96.98 (95.77–98.19)	2
Popo	94.55 (84.67–99.12)	7	96.28 (96.07–96.50)	2	95.37 (91.58–97.76)	6	96.48 (96.38–96.59)	2
Chloé	96.44 (93.95–98.02)	7	94.78 (92.61–95.92)	3	93.39 (87.74–97.32)	6	93.70	1
Pendesa	98.22 (97.66–98.57)	6	97.77 (96.59–98.38)	4	97.41 (96.00–98.22)	6	95.18	1
Iso-butyric acid[2]*, %*								
Pan	1.87 (0.81–2.71)	7	1.95 (1.54–2.20)	4	1.85 (1.35–2.57)	4	1.25 (0.78–1.73)	2
Popo	1.67 (1.17–2.68)	7	2.03 (1.67–2.40)	2	1.90 (1.45–2.44)	6	2.38 (1.98–2.77)	2
Chloé	1.60 (1.17–2.36)	7	2.00 (1.34–2.27)	3	1.52 (1.37–1.82)	6	3.12	1
Pendesa	1.62 (1.30–2.31)	6	1.93 (1.29–2.91)	4	1.44 (0.00–2.23)	6	2.81	1
Valeric acid[2]*, %*								
Pan	0.00 (0.00–0.00)	7	0.07 (0.00–0.27)	4	0.00 (0.00–0.00)	4	0.00 (0.00–0.00)	2
Popo	0.15 (0.00–0.49)	7	0.12 (0.05–0.19)	2	0.09 (0.00–0.16)	6	0.00 (0.00–0.00)	2
Chloé	0.05 (0.00–0.14)	7	0.08 (0.00–0.15)	3	0.20 (0.00–0.64)	6	0.25	1
Pendesa	0.05 (0.00–0.13)	6	0.00 (0.00–0.00)	4	0.15 (0.00–0.33)	6	0.00	1

Ranges are in parentheses. [1] Quantity obtained in 1 ml of ethanol. [2] Percentage of total acid quantity.

fatty acid was calculated by dividing it by the total amount of acids determined. The quantity of mucus was calculated as shown below, using the peak area of ethanol per 1 µl of injection:

$$\frac{\text{peak area of 30\% ethanol solution}}{\text{peak area of ethanol in sample}} - 1$$

Data Analysis

A two-way analysis of variance (ANOVA) was performed. One factor was the period (flat, partial swelling and early tumescence) and the other the individual chimpanzee (n = 4). Then we grouped the data in the flat, partial swelling and early tumescence into the non-ovulatory period when there was no significant effect of period and individual in the ANOVA. The non-ovulatory period and periovulatory period were compared with a t test, since there were insufficient data from each subject in the periovulatory period to consider the within-individual variance.

Table 3. The result of two-way ANOVA

Mucus or fatty acid		ANOVA	
		F value	p
Mucus	period	2.07	0.14
	individual	0.47	0.71
	interaction	2.03	0.08
Acetic acid	period	0.63	0.54
	individual	6.46	0.001
	interaction	0.67	0.67
Propionic acid	period	0.55	0.58
	individual	3.39	0.03
	interaction	0.45	0.84
Butyric acid	period	0.35	0.97
	individual	2.59	0 06
	interaction	0.96	0.46
Iso-butyric acid	period	1.24	0.30
	individual	0.80	0.50
	interaction	0.22	0.97
Valeric acid	period	1.13	0.33
	individual	2.47	0.07
	interaction	1.65	0.15

Results

Chemical Analysis

Acetic, propionic, butyric, iso-butyric and valeric fatty acids were identified (table 2). The quantity of mucus and percentage of fatty acids did not change during the three periods. The effects of individual on acetic acid and propionic acid were significant (table 3).

Compared to the non-ovulatory periods, the quantity of mucus tended to increase in periovulatory periods (t test: d.f. = 66, t = –1.99, p = 0.051). The percentage of iso-butyric acid tended to be higher in the periovulatory period, although the statistical test did not reach the 5% level (t test: d.f. = 66, t = –1.93, p = 0.058). Comparing the iso-butyric acid level in the early tumescence and periovulatory periods for each individual, the values of Chloé and Pendesa were higher in the periovulatory period than the range in the early tumescence period. No significant difference was seen for butyric acid (t test: d.f. = 66, t = 0.22, p = 0.83) or valeric acid (t test: d.f. = 66, t = –1.20, p = 0.24).

Behaviour

A male in Chloé's group (Reo) followed and frequently copulated with her when she began swelling in the sampling period. The same behaviour was seen in the next swelling period, and Chloé became pregnant. Such interactions were not seen between the males and the other three females.

Discussion

Chloé had the highest value of both acetic and propionic acids during the periods and she was the only female followed by a male and the only one to become pregnant. This suggests that acetic and propionic acids have a function in attracting males. Only the percentage of iso-butyric acid changed between the non-ovulatory period and periovulatory period. The values of two subjects were higher than the range in the early tumescence period. This implies that iso-butyric acid is a component of a chimpanzee pheromone or pheromone-like substance emitted near ovulation.

The results suggest that low-grade fatty acids from the vagina can be divided into two types: one type reveals individual differences throughout the menstrual cycle, e.g. acetic and propionic acids; the other type reveals no individual differences but changes in quantity according to the cycle, e.g. iso-butyric acid. If these acids function as pheromones, the former type might signal to males something related to the reproductive capacity of the females, while the latter type might inform the males of the physiological status of the females.

Acknowledgments

This study was supported by the Cooperation Research Program of Primate Research Institute, Kyoto University (to A.M.O. and Y.H.).

References

Fox GJ (1982). Potentials for pheromones in chimpanzee vaginal fatty acids. *Folia Primatologica* 37:255–266.

Goodall J (1986). *The Chimpanzees of Gombe.* Cambridge, Belknap/Harvard University Press.

Graham C (1981). Menstrual cycle physiology of the great apes. In *Reproductive Biology of the Great Apes* (Graham CG, ed.), pp 286–303. New York, Academic Press.

Hasegawa T, Hiraiwa-Hasegawa M (1983). Opportunistic and restrictive matings among wild chimpanzees in the Mahale Mountains, Tanzania. *Journal of Ethology* 1:75–85.

Jolly A (1967). Breeding synchrony in wild *Lemur catta*. In *Social Communication among Primates* (Altmann SA, ed.), pp 3–13. Chicago, University of Chicago Press.

Matsumoto-Oda A (1999). Female choice in the opportunistic mating of wild chimpanzees at Mahale. *Behavioral Ecology and Sociobiology* 46:258–266.

Michael RP, Keverne EB (1968). Pheromones in the communication of sexual status in primates. *Nature* 218:746–749.

Michael RP, Keverne EB (1970). Primate sex pheromones of vaginal origin. *Nature* 225:84–85.

Ochiai T, Matsuzawa T (1997). Planting trees in an outdoor compound of chimpanzees for an enriched environment. *Proceedings of the Third International Conference on Environmental Enrichment,* pp 355–364. San Diego.

Takasaki Y (1985). Female life history and mating patterns among the M group chimpanzees of the Mahale National Park, Tanzania. *Primates* 26:121–129.

Tutin CEG (1979). Mating patterns and reproductive strategies in a community of wild chimpanzees *(Pan troglodytes schweinfurthii). Behavioral Ecology and Sociobiology* 7:29–38.

Wallis J (1985). Synchrony of estrous swelling in captive group-living chimpanzees *(Pan troglodytes). International Journal of Primatology* 6:335–350.

Wallis J (1992). Chimpanzee genital swelling and its role in the pattern of sociosexual behavior. *American Journal of Primatology* 28:101–113.