A3 - Olfactory signals and fertility in zoo-housed Alaotran lemurs

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Purpose

The Lake Alaotra gentle lemur (Hapalemur alaotrensis) is one of the 25 most endangered primate species in the world. With a population continuously declining, it is estimated that only around 2,500 gentle lemurs remain in the wild (Reibelt et al., 2019).

This species, due to its high risk of extinction, is now targeted by several conservation projects (Reibelt et al., 2019), such as the Ex situ Programme (EEP). This population management programme is endorsed by European Association of Zoos and Aquaria (EAZA) members and aims to maintain captive healthy populations and maximize the genetic diversity through breeding management recommendations. Nevertheless, gentle lemurs are currently showing a low success rate in captive breeding across EAZA institutions. In this context, it is becoming increasingly important to further understand their reproductive biology.

Olfactory communication plays an important role in lemurs' socio-sexual behaviour (Charpentier et al., 2010; Scordato and Drea, 2007), and chemical signals have great potential as tools to trigger olfactory and sexual behaviours (Snowdon et al., 2006), which is remarkably important in critically endangered lemur species.

The aim of this study was to conduct the first detailed chemical analyses of vaginal secretions in gentle lemurs, including volatile compounds of odour signals in breeding and non-breeding periods, to identify the chemical signature conveying information about female sexual receptivity and fertility.

Design / Methodology / Approach

This study used non-invasive methods that integrate cutting-edge semiochemistry with behavioural observations and faecal endocrinology. Chemical investigation of vaginal odour was combined with the timing of fertility, estimated by using behavioural observations combined with faecal progesterone and oestradiol level

measurements, to evaluate cycle-dependent changes in female olfactory signals and their reliability as indicators of the fertile phase.

This research work focused on a captive family group, consisting of a breeding pair and their offspring (i.e. one subadult male and two infants) (n=5), of gentle lemurs hosted at Jersey Zoo over two periods (non-breeding period: August/September 2021; breeding period: December 2021/January 2022).

Behavioural data were collected from early morning to early afternoon (non-breeding period: 6h/day; breeding period: 4h/day) for 5 days per week (non-breeding period: Mondays to Fridays; breeding period: Sundays to Tuesdays and Thursdays to Fridays), for a total of 318 hours, using sampling all occurrences of some behaviours and ad libitum sampling methods (Altmann, 1974). Observations were focused on olfactory (brachial and ano-genital scent marking, sniff genitals), affiliative (grooming), sexual (ano-genital self-grooming, follow, mating calls, mounting attempt, copulation), and aggressive (intimidation) behaviours.

Faecal (n=54) and vaginal odour (n=35) samples of the breeding female were collected every morning. Samples were stored in a -20ºC freezer at the zoo and then transferred on ice to the Rosalind Franklin Science Centre, University of Wolverhampton, for laboratory analyses. Hormone levels were measured using Enzyme Immunoassay technique (Maréchal et al., 2011), while the volatile component of odour signals was investigated using solid-phase microextraction and gas chromatography-mass spectrometry (Walker and Vaglio, 2021).

Linear regression analysis was used to test the association between olfactory, sexual and affiliative behaviours and female estradiol levels during the two study periods. Statistical analyses were performed using R Studio software (version 4.1.1).

Findings

A significant positive correlation was found between oestradiol and female anogenital scent marks ($R^2 = 0.1815$, $F(1, 35) = 8.983$, $p = 0.005$), while male olfactory, sexual and affiliative behaviours were not significantly correlated with female hormone levels ($p > 0.05$), suggesting that male behaviour alone is not a clear indicator of female fertility.

Sexual and aggressive behaviours were observed occasionally, and only during the breeding period; in particular, copulation was displayed one day when occurred several times over an hour.

Hormone profiles showed a clear difference between the study periods (non-breeding vs. breeding). Particularly, during the non-breeding period the profiles of progesterone and oestradiol showed a synchronized pattern, but the trend did not indicate any ovulation, while during the breeding period two potential ovulation periods were identified, with an increase of oestrogen accompanied by a decrease of progesterone concentrations, interspersed by around 20 days. Sexual swelling and progressive opening of the vaginal orifice, as well as mating, were observed only during the second ovulation period. Thus, based on endocrinological and behavioural data, a two-day fertile window has been estimated.

A total of 56 volatile compounds, of which we tentatively identified 42, was detected in all female ano-genital odour samples, including a series of organic aliphatic acid esters, acetate esters, aldehydes, ketones, alcohols, terpenes, volatile fatty acids and hydrocarbons that have been identified in odour profiles of other primates. The vaginal odour profile changed over the study periods, with a small pool of volatile compounds which distinguished the breeding against the non-breeding period.

Research & Practical Limitations / Implications

Some major limitations have to be acknowledged for this work. First of all, the study focused on a small sample of subjects (i.e. one troop), due to the limited number of breeding pairs hosted by EAZA institutions. Additionally, because of the seasonality and the constraints related to zoo daily routine, the amount of daily sampling hours varied between the two study periods (i.e. breeding vs. non-breeding). Finally, less vaginal odour samples were collected during the non-breeding period due to the needed change of sampling protocol (i.e. from spontaneously released anogenital scent-marks to training for vaginal odour sampling). Nevertheless, this study improved our understanding of gentle lemur reproductive biology, and supported the hypothesis that fertile odour signals may play an important role in sexual communication and convey information about fertility and sexual receptivity in this lemur species.

Originality / Value

This novel project, co-designed with the gentle lemur EEP Coordinator and Studbook Keeper, has provided findings which can then be applied to captive gentle lemur management across European zoos in order to enhance the success of their captive conservation breeding programme. Particularly, based on this study findings a new scent enrichment will be designed, tested and implemented to trigger male mating behaviour and so improve reproductive success in breeding pairs of gentle lemurs. This study would then entail quantifiable impact, such as best practices adopted by zoos through amendments in management and husbandry policies released by the EAZA.

Link to SDG: As this project aims to enhance captive conservation breeding success in a critically endangered lemur species, it fits perfectly into the United Nations Sustainable Development Goal SDG 15 - Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss.

Keywords: captive conservation breeding, chemical signalling, faecal endocrinology, Hapalemur alaotrensis, reproductive biology.

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