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Ferret (*Mustela putorius f. furo*) odor affects the estrous cycle in Campbell's hamster females (*Phodopus campbelli*)

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Summary

Predator odors are known to repel prey species from e.g. feeding grounds and in some cases to have negative effects on the reproductive success of prey species. In the here presented study Campbell's hamster females were exposed to the urine odor of ferrets. Due to the odor exposure estrous cycles were severely disturbed, ovulations delayed or even inhibited. It is assumed that the sulphurous compounds in the urine are responsible for these effects, however, the underlying mechanisms are not known.

Zusammenfassung

Bekanntlich wirkt der Geruch von Raubsäugern abschreckend auf Beutetiere; sie werden etwa von Futterplätzen ferngehalten, und in einigen Fällen hat der Urin auch eine negative Wirkung auf den Fortpflanzungserfolg von Beutetieren. Diese Arbeit untersucht die Wirkung des Uringeruchs von Frettchen auf Weibchen des Campell-Hamsters. Infolge von Uringeruch wurde der Östrus-Zyklus der Weibchen empfindlich gestört; Ovulationen wurden verzögert oder sogar unterdrückt. Wir nehmen an, dass die Schwefel-Komponenten im Frettchenurin für diese Effekte verantwortlich sind, wobei aber der zugrunde liegende Mechanismus vorläufig unbekannt ist.

1. Introduction

In 1984 mustelid scent-gland compounds were found to suppress feeding in snowshoe hares (SULLIVAN and CRUMP 1984). Shortly afterwards predator odors (fecal, urine, and anal scent) were reported to be effective in suppressing damage

caused by various herbivorous species and, as a consequence of such findings, SULLIVAN et al. (1985) suggested the use of predator odors as repellents to reduce feeding damage by herbivores. In contrast to the many studies on feeding inhibition in prey species due to predator odor only few studies have tried to estimate the influence of predator odors on reproduction and population dynamics of their potential prey. For example it was shown that the odors of anal gland secretions of mink and weasel as well as stoat odor influence cycling in rats and voles (e.g. VOZNESSENSKAYA et al. 1992; KOSKELA et al. 1996). In a recent study VASILIEVA et al. (1999) demonstrated that cat urine affects sexual maturation and even meiosis in Campbell's hamster males (*Phodopus campbelli*). With the here presented study we wanted to investigate whether estrous cycles of Campbell's hamster females are affected in response to a simulated risk of small mustelid predation.

2. Material and Methods

2.1 Animals and housing conditions

Adult laboratory-born females were used in this study; the females were all of the same weight group; individual weight differences were less than 5 g at the beginning of our study. Prior to the odor exposure experiments all females had given birth to at least one litter. Animals were provided with fresh grains, seeds and vegetable and a pellet diet ad libitum. Shaved wood chips served as bedding. Animals were maintained on 14L:10D light cycle at $20\pm 2^\circ\text{C}$.

We randomly assigned females to either the control group (n=4) or to the exposure group (n=7). All animals were kept isolated from each other.

2.2 Determination of estrous cycle

To determine the estrous cycle we used the vaginal smear technique (WHITTEN and CHAMPLIN 1978;). This method is a reliable indicator of estrous, except in mammalian species with reflex or induced ovulations (MACFARLANE and TAYLOR 1982). In spontaneous ovulators like *Ph. campbelli*, a sequence of stages within the cycle is normally detectable when vaginal sampling is made at 24-h intervals. With this method the following four stages can be distinguished:

- diestrous*: the vaginal smear is largely mucus containing numerous leucocytes and only very few nucleated epithelial cells.
- proestrous*: abundance of large numbers of small, nucleated, epithelial cells; leucocytes are very rare.
- estrous*: the main cell type is cornified epithelium, other vaginal smear components are either minimal or absent.

metestrous: the smear is distinguished by massive leucocytes and large numbers of cornified epithelial cells.

Vaginal smears were obtained daily between 8.30 and 10.00 for three weeks (at least five estrous cycles). Cells were analyzed and counted using a Zeiss microscope.

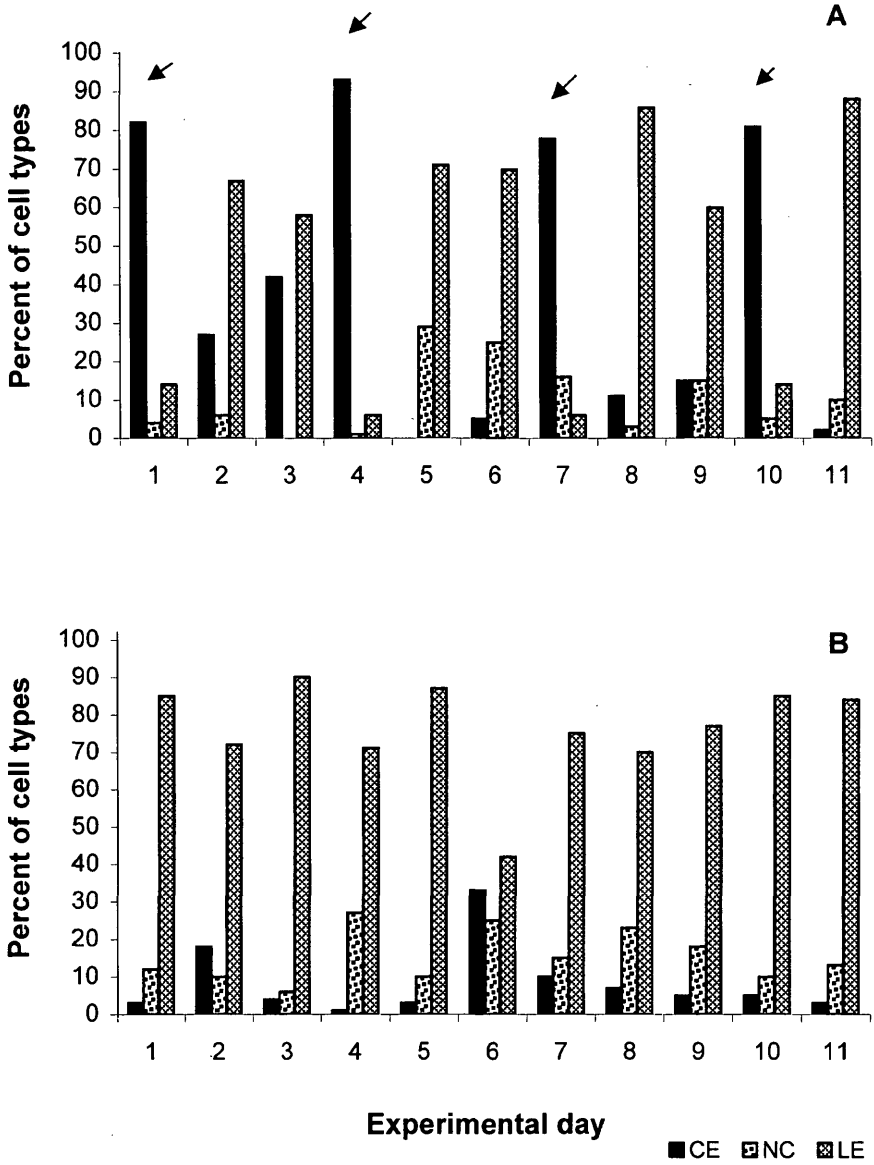
2.3 Odor exposure

All hamsters were predator naive before the beginning of the experiments. To simulate predation risk we kept the experimental females in close neighborhood of ferrets (*Mustela putorius f. furo*). In addition, approximately 0.5 ml of fresh ferret urine was applied all over the bedding material of the females daily. Control females were kept in close proximity and exposed to the odor of the related hamster species *Phodopus roborovskii*.

3. Results

In all control females a clear cut estrous cycle could be detected, in spite of the fact that the four stages mentioned above are somehow overlapping. Under our housing conditions mean duration of the estrous cycle was 4.08 days. However, small intraindividual variations occurred; interindividual variations in the mean duration were more obvious with a minimum of 3.6 days and a maximum of 4.3 days. Figure 1A depicts a typical estrous cycle of a control female.

In contrast to the control females the odor exposed females demonstrated massive disturbances in their estrous cycles. Already the first cycles after onset of the odor exposure experiments were affected: in 6 females the marked decline in the percentage of leucocytes after the metestrous stage did not take place. In addition the percentage of nucleated epithelial cells was fairly high as in the proestrous stage. In the remaining female percentage of leucocytes somehow decreased after the metestrous stage, as in control animals. Percentage of cornified epithelial cells remained low in all but one animals. The latter animal demonstrated almost normal cyclic changes with the exception that the mean duration (4 days in control females) extended to 6 days. After about 16 days three females returned to cyclic like changes in the composition of the vaginal smear, yet, the interindividual differences in the duration of the estrous cycles ranged between 6 and 7 days. Figure 1B depicts an example of a disturbed estrous cycle without an obvious ovulation.



4. Discussion

Predator odors are known to influence feeding behavior of several herbivore species and to reduce reproduction success in at least some prey species. As shown in the present study ferret urine odor severely affects the estrous cycles in Campbell's hamster females: cyclic changes do not occur as in control animals, ovulation seems inhibited or at least delayed. However, the underlying mechanisms are not known. Also not known are the active component or components in the predator urine. Some authors (e.g. NOLTE et al. 1994) suggested that sulphurous components or their metabolites in the urine as being responsible for these effects. In fact sulphurous compounds are present in the anal sac of the mink (*Mustela vison*) (SCHILDKNECHT et al. 1976; SOKOLOV et al. 1980) and in the anal secretion of the ferret (CRUMP 1980b); some of these compounds are also present in the stoat (*Mustela erminea*) (CRUMP 1980a). However, in a related study (ARNOULD et al. 1998) it was assessed which chemical constituents from dog feces are involved in its food repellent effect in sheep; the active odorous signals appeared to consist of fatty acids – not sulphurous components – mixed with neutral compounds acting synergically.

All laboratory experiments underline the complexity of these biological-chemical signals. Combined laboratory and field studies are needed to identify the components in urines, feces and/or anal sacs and to assess their effects on prey species development and behavior.

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Fig. 1: In control females (A) the different stages in the estrous cycle are characterized by changing percentages in the amounts of the cell types involved. Arrows indicate the estrous stages (ovulations). In experimental females exposed to ferret urine odor the characteristic changes no longer exist. In B the daily changes in the percentage of cell types of one female are given; there is no obvious estrous stage. CE = cornified epithelial cells; NC = nucleated epithelial cells; LE = leucocytes.

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