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The individual courtship behaviour of male European mink (*Mustela lutreola*) is a good indicator for their breeding success

Nadja Kneidinger[a,*], Astrid Nagl[a], Kairi Kiik[b], Franz Schwarzenberger[a], Tiit Maran[c,d]

[a] Department of Biomedical Sciences – Physiology, Pathophysiology and Experimental Endocrinology, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria
[b] Department of Zoology, Institute of Ecology and Earth Sciences, Tartu University, Vanemuise 46, 51014 Tartu, Estonia
[c] Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 62, 51014 Tartu, Estonia
[d] Species Conservation Lab, Tallinn Zoological Gardens, Paldiski Road 145, 13522 Tallinn, Estonia

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ABSTRACT

Conservation breeding of the critically endangered European mink (*Mustela lutreola*) is a challenge, because the genetically prioritised breeding pairs often fail to mate during the staged breeding attempts. For this study the courtship and mating behaviour of 33 males and the response behaviour of 28 females was observed and recorded throughout two breeding seasons at Tallinn Zoo using an ethogram. The behavioural patterns of the male European mink differed significantly depending upon their previous reproductive success and the outcome of the breeding attempt. "Clucking", "flehmen", "chase", "anal drag", "neck bite" and "mounting" were observed significantly more often among males that sired a litter during the study ("breeding males") and during attempts that ended with copulation. More than half (61%) of the males were "non-breeding males": they never sired a litter or copulated with a female. Females "chirped" significantly less often in attempts with "non-breeding males". The male "clucking" sound was the best indicator to predict 1) a males ability to sire a litter and 2) whether a breeding attempt would end with copulation. The absence of species-specific courtship and mating behaviour in the "non-breeding males" threatens the conservation goal to maintain the genetic heterozygosity of the captive population. From a practical point of view, behavioural indicators could be used as a potential screening method for identifying successful future breeders. In an experimental setup, males were presented with scent cues from an oestrous female but without the opportunity for direct contact. In this experimental context 64% of "breeding males" responded with "clucking" and also presented "flehmen", "rubbing" and "approach to the female/her nest box door" significantly more often than the "non-breeding males".

1. Introduction

The European mink (*Mustela lutreola*) was listed in the IUCN Redlist as "vulnerable" in 1988 and as "critically endangered" in 2011. From its former distribution all over continental Europe only isolated fragments remain in northern Spain and western France, in the Danube delta in Romania and in Ukraine and Russia. The rate of decline continues and is predicted to exceed 80% within the next ten years (Maran et al., 2016). For 25 years the captive breeding of the European mink has been conducted in the frame of an EEP programme (European Endangered Species Programme) supervised by the European Association of Zoos and Aquaria (EAZA) and coordinated by the Tallinn Zoological Gardens in Estonia. The main goal of the EEP breeding programme is to maintain 85% of the original heterozygosity of the captive population for 50 years.

Captive breeding at Tallinn Zoo has been highly successful, with a sufficient number of litters produced every year to maintain the captive population at the Zoos full capacity and for ongoing reintroduction projects into the wild. Nevertheless, maintaining the genetic heterozygosity of the captive population is an ongoing struggle. Breeding pairs specifically selected according to their genetic representation among the population do not always mate during the breeding attempts, yet positive outcomes are critical to prevent inbreeding. Kiik et al. (2013) found that over a six year period, only 25% of breeding attempts in Tallinn Zoo resulted in copulation. An endocrine study by Nagl et al. (2015) determined that breeding failure is not caused by dysfunction of the reproductive cycle of the females. All females in the study group had physiological oestrous cycles and pregnancies.

Two variants of abnormal mating behaviour of the males, passivity and aggressiveness, were the strongest indicators of breeding failure

* Corresponding author.
E-mail address: nadjakn@gmx.at (N. Kneidinger).

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(Kiik et al., 2013). Knowledge of the normal breeding behaviour is an essential prerequisite to identify the causes of breeding failure on a behavioural level (Lindburg and Fitch-Snyder, 1994). For instance the success of the current breeding programme for the captive giant panda (*Ailuropoda melanoleuca)* population has been greatly improved by the analysis of the reproductive behaviour and subsequent adaptations of the breeding management (Zhang et al., 2004).

The breeding behaviour of the European mink has not been described so far, but there are detailed descriptions for other closely related mustelid species, the European polecat (*Mustela putorius;* Davison et al., 2000) and the domestic ferret (*Mustela putorius furo;* Poole, 1972). The aims of the present study were:

1) to characterise the courtship and mating behaviour of the male European mink during controlled breeding situations, and to establish an ethogram; 2) to compare and to distinguish the male's reproductive behavioural repertoire in successful versus failed breeding attempts; 3) to characterise the female's response during breeding situations and possible correlations to the outcome of the mating attempts; 4) to assess whether chemical cues could provide a way of screening for effective breeders in advance, by categorising the behaviour of male European mink in response to scent marks left behind by a female in oestrus.

2. Material & methods

2.1. Study population and housing

The European mink observed during this study were part of the captive population (n = 100 to 120 animals) kept at the Species Conservation Research Lab at Tallinn Zoological Gardens, Estonia. This Research Lab is a part of the Zoo and is not open to the public. The animals were captive born and had individual housing in adjacent enclosures (size 2 m × 2 m × 4 m) fenced off by plywood and mesh wire. The animal houses each hold two rows of ten enclosures. Most of the animals had visual contact to the opposite sex. The enclosures are outdoors and exposed to natural weather conditions. All enclosures were equipped with a nest box that had hay bedding for insulation and could be used to trap the animals and to transfer them to a small cage for handling. Standard housing items were a swimming pool and objects for environmental enrichment that were replaced every few weeks (logs, branches, tubes, small trees, dog toys). Feeding time was in the morning; on some days extra rations were given at noon. The enclosures were cleaned from faecal matter and leftover food every day, and the housing equipment was readjusted.

2.2. Ethogram for the observation of breeding behaviour

Based on the descriptions by Poole for polecats (*Mustela putorius* and *M. putorius furo* x *M. putorius hybrids;* 1967, 1972 and 1974), Vargas and Anderson for black-footed ferrets (*Mustela nigripes;* 1998), and Pedersen et al. for farmed mink (*Mustela vison;* 2004), we compiled an ethogram for the observation of breeding behaviour of the European mink (Table 1). During a pilot study the most relevant behavioural elements were identified and 22 predefined elements were listed on an observation sheet. Fifteen elements describe behavioural patterns shown by males, five elements describe female behavioural patterns and two elements (vocalisations) can be observed both in males and females.

2.3. Animals and captive breeding management

In the two study years the goal of the captive breeding programme was to deliver 34 litters. Of all European mink kept at Tallinn Zoo, 28 females (age range: 1–7 years) and 33 males (Table 2; age range: 1–6 years) were included in the breeding attempts. Six females and nine males took part in both years. Preparation prior to the breeding seasons included the identification of the genetically most suitable breeding partners (Software: Population Management 2000). Females selected for breeding were regularly checked for signs of oestrus by measuring vulva size and the percentage of cornified cells in vaginal swabs (see Nagl et al., 2015) from early March onwards. Females in oestrus were matched with the most suitable male. This male was introduced to the female's enclosure and if copulation was achieved the male remained with the female for 3–7 days. Subsequently the female was expected to give birth after 42 days. Aggressive behaviour by the male towards the female prompted its immediate removal from the enclosure. If the primary mate failed, the genetically second best suited male for this female was determined. Depending on how many females were scheduled for breeding on a certain day, the new male was either introduced right away or after the attempts with the other females had been completed. No more than three different males were attempted with the same female on one day. If no copulation was achieved, the mating attempts were continued during subsequent oestrus periods. During one breeding season (March until the beginning of May) nonbred females repeatedly came into oestrus and several pairings could be tested.

2.4. Observation of breeding behaviour. I. 'Natural breeding' in the captive environment

The male was brought to the designated female's enclosure in a transport cage and released. The observer immediately left the enclosure and stepped into the adjacent enclosure of an animal on either side to start the observation. An observation sheet with the 22 behavioural elements established in the ethogram was used for all-occurrence focal-animal sampling (Altmann 1974). The observer recorded all occurrences of every given behavioural element for the duration of the mating attempt. Maximum duration of an attempt was one hour. Attempts were terminated earlier when the breeding pair copulated or had to be separated due to adverse behaviour of the male or the female. Altogether n = 120 breeding attempts were observed during the two study years.

2.5. Observation of breeding behaviour. II. 'Experimental setups'

Experimental setups were used to assess whether chemical cues could provide a way of screening for effective breeders in advance. Thirty-one of the 33 male European mink were exposed to an experimental situation. Additional females were selected from the population to ensure the males had not had any direct contact to the same female during that breeding season.

During the experimental setup the males were allowed to investigate the cage of a female in oestrus, while the female was locked into the adjoining nest box. The observer would enter a neighbouring cage to start the observation immediately after releasing the male into the enclosure. The observation was limited to the behavioural elements that did not require direct contact to the female: "active approach to the female" or in this case, the closed nest box with the female inside; "chirp" vocalisations uttered by the female in the nest box; "clucking" vocalisations of the male; "flehmen", "foraging", "intensive sniff", and the marking behaviours "anal drag", "defecating", and "rubbing". The behavioural elements were recorded as all-occurrence focal-animal sampling for the duration of ten minutes.

2.6. Data analysis

The males were grouped according to their breeding success; males that mated with a female and sired a litter during the course of the study were categorised as "breeding males"; otherwise the category was "non-breeding males". One male successfully achieved copulation once, but did not sire a litter and was excluded from this comparison. The males were exposed to varying numbers of breeding attempts; the correlation between the number of mating attempts and the occurrence of behavioural elements was tested by Linear Regression. For further

Table 1
Ethogram describing the behavioural elements included in the final breeding protocols.

Behaviour	Description
Active approach to markings	Advance towards substances e.g. urine or faeces left by another individual or towards the site of former occupation by another individual.
Active or intentional approach	Advance towards another individual. In experimental context advance towards nest box where the other individual is confined.
Anal sniff	Sniffing beneath the base of the tail (Poole 1967).
Attack	Agonistic behaviour intended to frighten or damage another individual (Pedersen et al., 2004).
Chase	Pursuing another individual.
Chirp	Vocalisation by the female outside the context of chase or intimidation.
Clucking	Described by Poole (1972) as repeated cluck, cluck, cluck. Generally made by a confident or excited individual, never by an intimidated one. Shown also by mothers interacting with their pups.
Copulation	A long lasting (> 1 min) mount accompanied by pelvic thrusts, is possible only with a passive partner (Poole 1974).
Fake flank bite	Running alongside another individual while delivering brief biting motions in the direction of the flanks. These bites rarely make physical contact.
Flehmen (visible licking)	A form of chemosensory investigation of a site or substance of particular interest that exposes the vomeronasal organ (Mellen 1993; Swaisgood et al., 2002; Allen et al., 2016). In the European mink flehmen is characterised by visible licking, either hovering above or directly at the site or substance.
Foraging	The animal walks around in the enclosure, sniffing and investigating the surroundings (Pedersen et al., 2004).
Hiss	Vocalisation uttered as a threat (Poole 1972).
Immediate attack	Agonistic behaviour shown immediately after visual contact with another individual.
Intensive sniff	Audible nasal investigation of substances (Stahlbaum and Houpt, 1989).
Marking behaviour: Anal drag	Rubbing anal area against the floor or furniture in the cage.
Marking behaviour: Defecating	Evacuating waste matter from the bowels (Vargas and Anderson 1998).
Marking behaviour: Rubbing	Rubbing of flanks or abdomen along the floor, wire mesh or furniture in the cage.
Mounting	Straddling another animal with the fore paws held on either side of the flank whilst the neck is held dorsally or dorsolaterally in the teeth. The hind paws usually rest on the ground (Poole 1972).
Neck bite	Gripping with the teeth the side or back of the neck in the region behind the ear and anterior to the shoulder (Poole 1972).
Scream	Vocalisation in the context of discomfort or fear (Poole 1972).
Stereotypies	The only stereotypy observed was pacing: The animal repeatedly runs back and forth along the length of the cage (Pedersen et al., 2004).
Tail bite	Grabbing the tail of another individual with the teeth, sometimes dragging it.

Table 2
List of all males included in the breeding plans during the two study years and their success rate (number of copulations and/or litters).

	1st study year			2nd study year		
Male	Age (yrs)	Mating attempts	Copulations/Litters	Age (yrs)	Mating attempts	Copulations/Litters
Ants	(4)	4	**3/2**	*no mating attempts*		
Joel	(2)	2	**2/2**			
Priit	(1)	1	**1/1**			
Rami	(6)	1	**1/1**			
Gandalf	(1)	2	**n**			
Hantrus*	(2)	2	**n**			
Lorbert	(1)	2	**n**			
Mardus	(2)	1	**n**			
Nemo	(1)	2	**n**			
Nöps	(1)	1	**n**			
Popi	(1)	1	**n**			
Semu	(2)	1	**n**			
Värdi*	(7)	2	**n**			
Finn	(4)	3	**2/2**	(5)	3	**2/2**
Madis	(3)	2	**1/1**	(4)	3	**1/1**
Pernod	(3)	3	**3/2**	(4)	2	**2/2**
Rünnar	(2)	4	**1/1**	(3)	3	**1/0**
Armin	(3)	5	**1/1**	(4)	4	**n**
Dim*	(4)	2	**n**	(5)	9	**n**
Glenn	(1)	1	**n**	(2)	2	**n**
Kepp	(1)	1	**n**	(2)	6	**n**
Ged	*no mating attempts*			(3)	3	**3/2**
Poolakas				(3)	5	**4/2**
Rinaldo				(2)	7	**3/3**
Morten				(1)	1	**1/0**
Edgar				(2)	4	**n**
Elton				(2)	3	**n**
Jaak				(3)	1	**n**
Leemet				(3)	3	**n**
Leib*				(4)	2	**n**
Remsu				(2)	7	**n**
Roi				(2)	5	**n**
Triip*				(6)	4	**n**

* … this male has sired a litter in earlier years.
n … no copulation or litter.

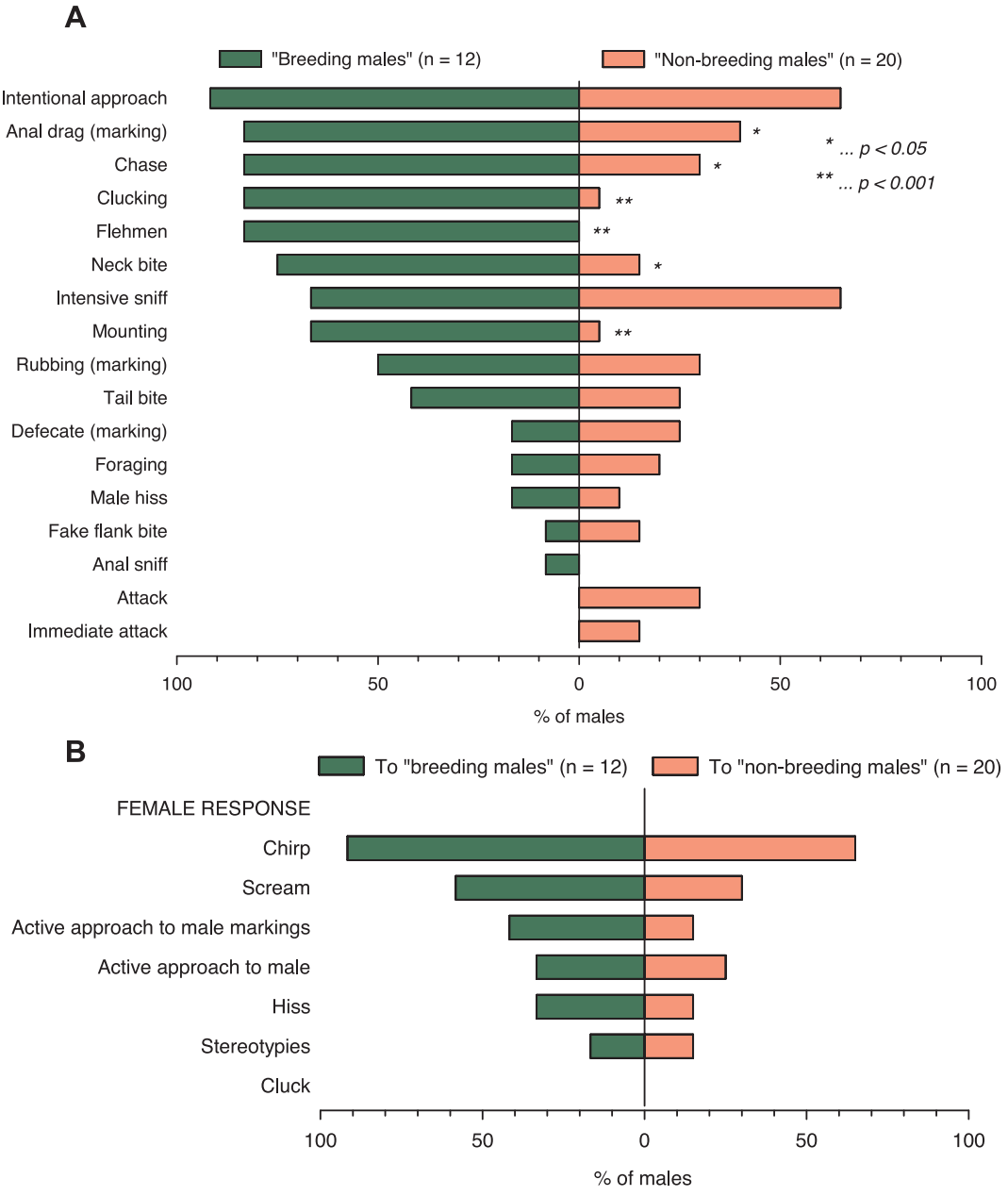

Fig. 1. (a) Comparison of the percent of males of each group ("breeding males" vs. "non-breeding males") that displayed each of the behavioural elements during the first recorded observation. Significant differences between the groups are indicated ($p < 0.05$; $p < 0.001$). (b) Comparison of the femalés responses to "breeding males" and "non-breeding males". No significant differences were found.

Table 3
Of the behavioural elements that had a statistically significant difference in the occurrence between "breeding males" and "non-breeding males", two showed a positive correlation to the number of mating attempts. The p values and adjusted r^2 from Linear Regression are presented for each group.

	Anal drag		Clucking	
	p value	adj r^2	p value	adj r^2
"Breeding males"	< 0.001	0.728	< 0.001	0.758
"Non-breeding males"	< 0.001	0.614	–	

statistical analysis only the first attempt of each male was considered to exclude possible bias. The ratio of males in each group that displayed the behavioural elements ("clucking", "flehmen", i.e.) was compared by Fisher Exact Test. The mean and standard deviation of the duration of the observations were calculated for the "breeding males" and the "non-breeding males". For the analysis of the experimental setups again only the first setup was included for each male because several males were observed only once. Statistical differences between "breeding males" and "non-breeding males" were determined by Fisher Exact Test.

The breeding attempts were categorised based on the occurrence or non-occurrence of copulation. The attempts that did not end in copulation featured either "breeding males" or "non-breeding males" and were therefore subdivided again. In these categories the number of breeding attempts per male varied. First the percentage of breeding attempts during which the behaviour had been observed was calculated for each male individually. Then the mean percentage was calculated for each of the three categories. Significant differences between the categories were determined by One Way ANOVA (Analysis of Variance) on Ranks, and All Pairwise Multiple Comparison Procedures (Dunńs Method) was used as a post hoc test.

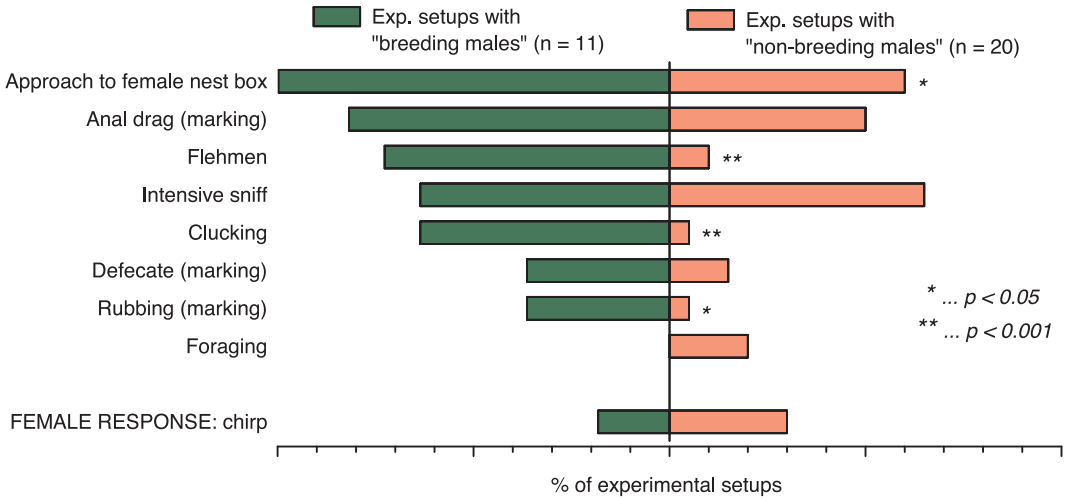

Fig. 2. Comparison of the behavioural elements that were recorded during experimental setups. Behavioural elements were observed significantly more often among "breeding males", they are indicated ($p < 0.05$; $p < 0.001$). The female was confined in the nestbox, but her vocalisation "chirp" was recorded.

For each category the mean and standard deviation of the durations of the attempts was calculated. The duration of the observations varied due to: the outcome (copulation/no copulation); severe male or female agonistic behaviour; environmental conditions; restrictions due to the conservation breeding protocols. The occurrence of behavioural elements during attempts that lasted 0–30 min and attempts that lasted 31–60 min was calculated and tested for statistically significant differences by Chi-square analysis of contingency tables. The calculations were performed with WPS Spreadsheets (WPS Office 2016), the statistical analyses with SigmaPlot (Systat Software 2011, version 12.3).

The behavioural data was transferred to SPSS (IBM, version 17.0) for Principal Component Analysis (PCA) and Classification and Regression Trees (CRT). PCA was applied to reduce the amount of data by identifying a clustered occurrence of certain behavioural elements. Each of the individual behavioural elements and the resulting categories from the PCA were analysed in CRTs for their suitability to predict 1) whether a male sired a litter during the two study years, and 2) whether copulation would occur during a specific breeding attempt. The specifications used for CRT were: growing method = CHAID (Chi Square Automatic Interaction Detection), maximum tree depth = 3, minimum cases in parent node = 10, minimum cases in child node = 5.

3. Results

3.1. Breeding behaviour

Several elements of breeding behaviour were observed among a significantly larger fraction of "breeding males" than "non-breeding males" (Fig. 1a): "clucking", "flehmen" and "mounting" (each $p < 0.001$); "chase", "neck bite" and "anal drag" (each $p < 0.05$). Female responses did not differ significantly between the two groups (Fig. 1b). The average duration of the observations was 37 ± 25 min (mean ± SD, range 7–60 min) for the "breeding males" and 44 ± 19 min (mean ± SD, range 15–60 min) for the "non-breeding males". For the majority of the behavioural elements Linear Regression showed no positive correlation between the occurrence of the behaviour and the number of breeding attempts. Of the behavioural elements that were seen significantly more often in "breeding males", "clucking" had a positive correlation among "breeding males" but not among "non-breeding males". "Anal drag" had a positive correlation for both "breeding males" and "non-breeding males"; p values and adjusted r^2 values are listed in Table 3.

3.2. Breeding behaviour during experimental setups

"Clucking", "flehmen" (each $p < 0.001$), "rubbing (marking)" and "approach to female nest box" (each $p < 0.05$) were observed significantly more often in experimental situations that featured "breeding males" (Fig. 2).

3.3. Differences in breeding behaviour depending on the outcome of the breeding attempt

Of 120 observed breeding attempts n = 32 ended with copulation (featuring n = 13 males). The attempts that did not end in copulation featured either "breeding males" (n = 24 attempts, featuring n = 7 males) or "non-breeding males" (n = 64 attempts, featuring n = 20 males). A different repertoire of breeding behaviour was observed during the breeding attempts depending on whether copulation took place and whether a "breeding male" or a "non-breeding male" was involved. The occurrence of several behavioural elements differed significantly ($p < 0.05$ or $p < 0.001$, Fig. 3a and Table 4). Courtship behaviour "clucking" and "flehmen" was displayed significantly more often by "breeding males", regardless of whether the attempt ended with copulation. Attacks towards the females were almost only seen during attempts that did not end in copulation.

Attempts that ended in copulation were the shortest and averaged at 23 ± 22 min (mean ± SD, range 4–60 min). Attempts with "breeding males" that did not end with copulation lasted 43 ± 16 min (mean ± SD, range 8–60 min) and attempts with "non-breeding males" went on for 40 ± 18 min (mean ± SD, range 4–60 min). When comparing the attempts that lasted up to 30 min to attempts that continued for 31–60 min, the majority of behavioural elements showed no significant difference in occurrence between the groups (Fig. 4). Copulation was observed more frequently among the shorter attempts. The behavioural elements "defecate" and "tail bite" occurred significantly more often during the longer attempts ($p < 0.05$).

3.4. Female response behaviour

Regarding the female behaviour there were no differences in the occurrence of the responding behavioural elements "scream, hiss, cluck, stereotypical behaviour, approach to male markings, approach to male" during breeding attempts with "breeding males" compared to attempts with "non-breeding males" (Fig. 1b). In breeding attempts that did not end with copulation the vocalisation "chirp" was observed significantly more often when a "breeding male" was present compared to attempts

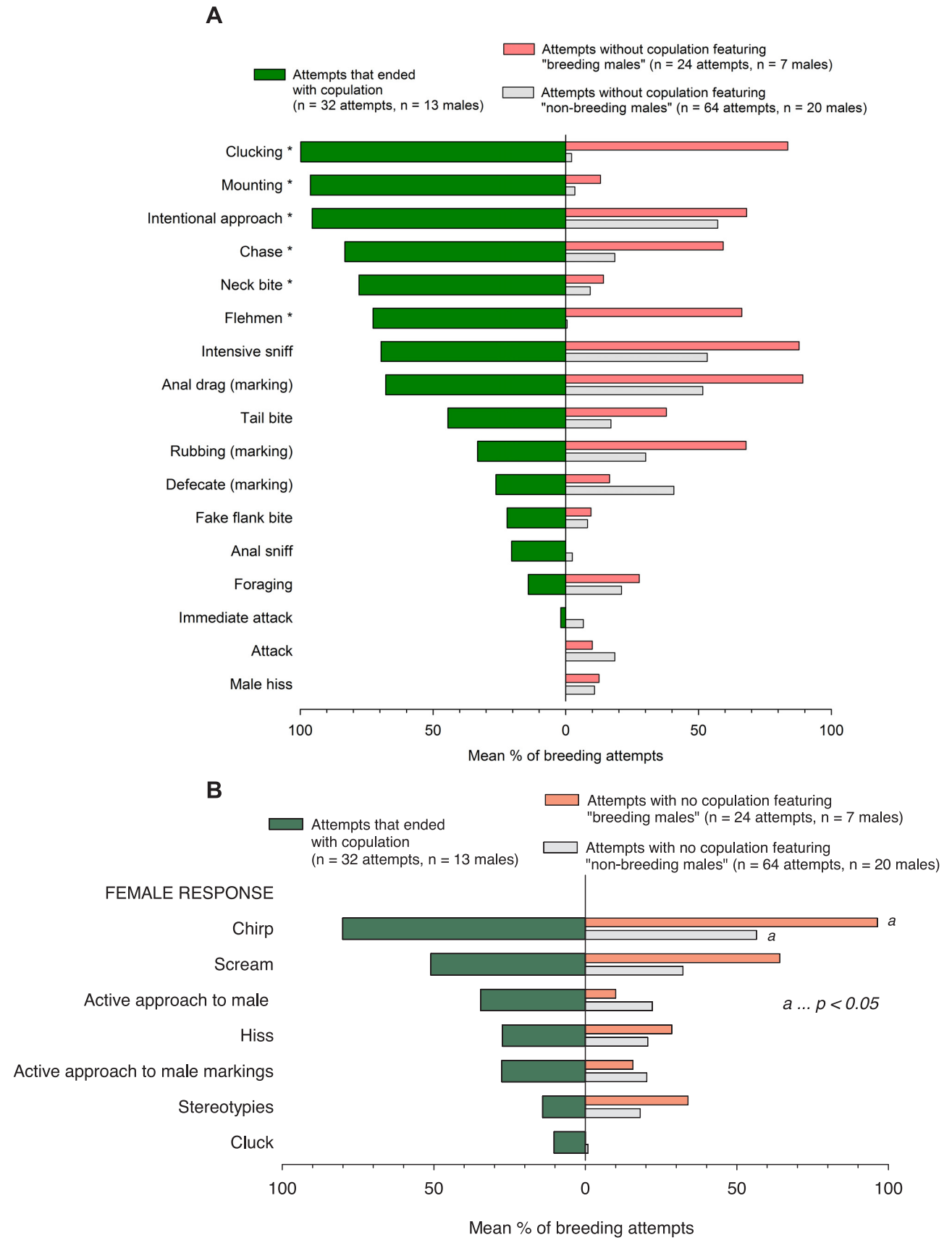

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Fig. 3. (a) The occurrence of behavioural elements according to the outcome of the breeding attempt: attempts that ended with copulation compared to attempts that did not result in copulation. The latter (on the right side) are subdivided depending on whether it featured a "breeding male" or a "non-breeding male". Behaviour with significant differences between the groups is indicated in the graph (*), and the p values are listed in detail in Table 4. (b) A comparison of female responses in dependence of whether an attempt ended with copulation, and whether a "breeding male" or a "non-breeding male" was present during the attempts that did not end in copulation. The vocalisation "chirp" was recorded significantly more often during attempts with "breeding males" compared to "non-breeding males", regarding the attemtps that did not end in copulation ($p < 0.05$).

Table 4
Results of All Pairwise Multiple Comparison Procedures (Dunńs Method) for significant differences in the occurrence of behavioural elements between the categories (see Fig. 3a & b).

	Attempts with copulation x Att. w/o copulation feat. "breeding males"	Attempts with copulation x Att. w/o copulation feat. "non-breeding males"	Att. w/o copulation feat. "breeding male" x Att. w/o copulation feat. "non-breeding males"
Clucking		$p < 0.01$	$p < 0.01$
Mounting	$p < 0.01$	$p < 0.01$	
Intentional approach		$p < 0.01$	
Chase		$p < 0.01$	
Neck bite	$p < 0.05$	$p < 0.01$	
Flehmen		$p < 0.01$	$p < 0.01$
Female chirp			$p < 0.05$

Footnote: Att. = Attempts w/o = without feat. = featuring.

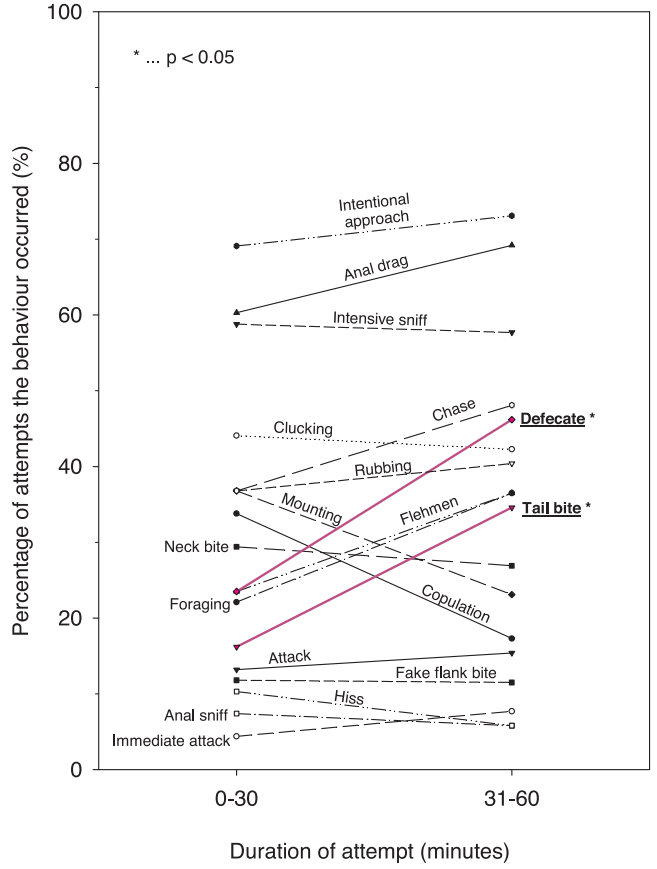

Fig. 4. The occurrence of behavioural elements compared between attempts that lastet up to 30 min and attempts that lasted 31–60 min. The connecting lines indicate whether the percentage increased or decreased between the groups. Elements with significant differences are indicated ($p < 0.05$).

with "non-breeding males" (Fig. 3b; $p < 0.05$).

3.5. Principal component analysis (PCA) and correlation regression trees (CRT)

The behavioural elements with sufficient Kaiser-Meyer-Olkin Measures of Sampling Adequacy were included in the PCA (KMO value = 0.829). Four components explained a total variance of 65.8%. In the first component "clucking", "flehmen", "neck bite", "mounting" and "copulation" were grouped together (percentage of variance = 28.7%). The second component grouped "fake flank bite", "tail bite", "chase" and "intentional approach", while the third component grouped "rubbing", "anal drag" and "intensive sniff". "Anal sniff" stood alone as the fourth and weakest component. Both the PCA components and each behavioural element on its own were used for CRT. The strongest result from the CRT analysis was received when using the behavioural element "clucking" as a single predictor. Whether a litter had been sired during the study or not was predicted with 90.8% accuracy. The precision was 87.3% for "litter sired" and 93.8% for "no litter sired". Copulation could be predicted with 83.3% accuracy, with a 100% correct classification of the breeding attempts that ended with copulation, and a 77.3% correct classification of the breeding attempts that ended with no observed copulation. PCA and CRT were also attempted for the behavioural elements observed during experimental situations, but the data did not pass the statistical tests for sampling adequacy.

3.6. Assessment of the breeding attempts and litters

Of 34 scheduled litters 23 were delivered. The litters were sired by only 12 males ("breeding males"; 36% of the males taking part in this study). One male copulated but this did not result in a litter. The rest of the males did not achieve copulation in any of the breeding attempts and did not sire any litters ("non-breeding males", n = 20; overview see Table 2). The majority of the males were observed during several breeding attempts ("breeding males": mean ± SD = 3.2 ± 2.6, range 1–9; "non-breeding males": mean ± SD = 4.3 ± 2.6, range 1–11). On average it took 3.5 ± 2.1 (mean ± SD) attempts with different and/or the same males (for examples see Table 5) until copulation was observed (mean ± SD = 2.6 ± 1.7 attempts in the 1st year of study, mean ± SD = 4.5 ± 2.0 attempts in the 2nd year of study). After several attempts with older females no litter was delivered even though copulation was observed. These females were six years (n = 3) or seven years (n = 2) old. During this study no seven year old female produced a litter. One breeding attempt with a young female (two years old) ended with copulation but for no apparent reason no litter was delivered. One female died during the gestation period (six years old). In two cases where the breeding attempt was repeated on the next day and the breeding pair copulated both times, a litter was delivered by the female.

4. Discussion

The present study found significant differences in the behavioural patterns of male European mink (*Mustela lutreola*) depending upon their reproductive success. The courtship and mating behaviour related to mating success was analysed – specific behavioural elements were observed in males that sired a litter during the study ("breeding males"): "clucking", "flehmen", "chase", "anal drag", "neck bite" and

Table 5
Representative examples for the sequences of mating trials and the males attempted with each female. The males in bold are those that were attempted last and in case of copulation sired the litter. 1) Female "Absinth": a single breeding trial resulted in copulation and a litter was delivered. 2) Female "Mari": several males were tested. Only the last, "Ants", sired a litter. 3) The 6 year old female "Karlutt" copulated with the male "Ants" during the 2nd attempt, but no litter was delivered. 4) Several males were tried with the female "Rändi", but none of them mated with her. Finally a proven breeder, "Armin", was introduced to the female, but copulation again was not observed and she delivered no litter.

Female	Age (yrs)	Mating attempts	Copulation/Litter (0 … no; 1 … yes)	*Male breeding partners in chronological order*						
Absinth	(3)	1	**1/1**	**Armin**						
Mari	(4)	4	**1/1**	Hantrus	Hantrus	Semu	**Ants**			
Karlutt	(6)	3	**1/0**	Kepp	Ants	**Ants**				
Rändi	(1)	7	**0/0**	Dim	Värdi	Dim	Gandalf	Värdi	Armin	**Armin**

"mounting". Significantly less of the "non-breeding males" expressed these behavioural elements. There was no difference in the behavioural patterns of the females between attempts with "breeding males", regardless whether they ended with copulation or not. Males were only introduced to females when oestrus had been detected, which might explain the lack of difference in the female response. During breeding attempts that did not end in copulation the female vocalisation "chirp" was detected significantly less often among the attempts with "non-breeding males", possibly because it was less common for these males to approach or chase the females. The females usually stay hidden unless the males intentionally approach them. Females were not categorised according to whether they had any litters, as all but one female included in the study had at least one litter during, before or after the two study years.

Kiik et al. (2013) observed that breeding success depended more strongly on the male than on the female partner, although they did not investigate whether the female played a role regarding the malés behaviour. Lodé (1991) described how body contact, olfactory investigations of the femalés body and the "cluck" of the male Stone marten (*martes foina*) seemed to inhibit an aggressive response by the female. The "clucking" sound has also been described in other mustelids such as the polecat (Poole, 1972). The present study revealed that mating attempts during which the European mink male did not "cluck" never ended with copulation. "Breeding males" also clucked in 83% of breeding attempts that did not end with copulation, indicating that the expression of this behaviour is part of the courtship phase prior to mating. Analysis by Classification and Regression Trees (CRT) confirmed that "clucking" is the strongest element to predict copulation and breeding success with high accuracy. This result may have been influenced by the weather conditions during the observations. In snowy conditions the animals are sometimes hidden from view, leaving only the vocalisation for the observer to be distinguished at all times. "Flehmen" is also part of the initial courtship behaviour and was displayed significantly more often by "breeding males". "Flehmen" in mammals is performed as a function of chemosensory analysis of sexually significant materials (Hart, 1983). Chemical cues play a role especially in solitary and seasonally mono-estrous species like the giant panda (Swaisgood et al., 2002). A mustelid species in which the reaction to specific odours was tested, is the ferret (*Mustela putorius f. furo*); males of this species show great interest in urogenital and body odour of opposite-sex conspecifics during the breeding seasons (Berzins and Helder, 2008).

During the experimental setup of the present study the "breeding males" displayed elements of breeding behaviour significantly more often in response to the odours in the enclosure of an oestrous female: "clucking", "flehmen", "rubbing" and "intentional approach to the female". The female was separated in the adjoining nest box and not in the enclosure with the male. This suggests that the European mink relies upon chemical cues in a reproductive context. Exposing the European mink males to the odours of a female in oestrus in an experimental setup might offer an opportunity to screen for effective breeders in advance, without risking injuries to the females or genetically unsuitable copulations. Before implementation of this concept as a tool for breeding, a follow up study should be conducted on young males that have not been exposed to females before, including an assessment of their breeding success in later life.

Agonistic behaviour towards the female was seen both in attempts with "breeding males" and "non-breeding males", but almost never during attempts that ended in copulation. With *ex situ* breeding, it is often difficult to judge whether an attack is part of the normal courtship behaviour or may lead to a severe injury of the female. The expression of non-aggressive courtship behavioural patterns provides good guidance in making such a prediction.

Both male aggressiveness and passivity have been identified as two expressions of abnormal behaviour and as primary causes of breeding failures in European mink (Kiik et al., 2013). The results of this study support the conclusion that breeding failure is caused by behavioural issues of the males, not by issues concerning the reproductive physiology. This is an interesting contrast to findings such as in the black-footed ferret (*Mustela nigripes*), where pathological sperm was described as one of the reasons for breeding failures in captivity (Wolf et al., 2000).

The European mink males of our study that copulated with a female almost always sired a litter. The few exceptions were due to old age or even death of the female. On the other hand more than half of the males observed during this study failed to copulate with a female. During the two study years only approximately one third of the planned litters were sired by the genetically prioritised males. This jeopardises the goal to maintain the heterozygosity of the captive population, as the genes of these "non-breeding males" are not passed on. A few of them had reportedly sired litters in earlier years, indicating that the captive environment (or management) might have had some adverse effects on their breeding behaviour over time. A study on the reproductive behaviour in wild European mink does not exist, but all of the European mink caught in the wild that were used as founders for the population at Tallinn Zoo sired litters. The director of Tallinn Zoo just recently reported of a one-year old male European mink that was born in the wild, recaptured in 2016 and successfully copulated with females during the next breeding season (T. Maran, personal communication, April 16, 2017). A study by Díez-León et al. (2013) found that American mink males reared in barren environments copulated less than conspecifics that lived in adapted, enriched home cages. The European mink males of our study population are all housed in identical enriched enclosures, but the emergence of "non-breeding males" suggests there is another yet unknown factor that has a strong detrimental effect on the development of reproductive behaviour. Lindburg and Fitch-Snyder (1994) described that deficient early rearing environment can cause behavioural inadequacies affecting reproductive performance. One obvious difference between a captive environment and the wild is that the time of weaning is artificially scheduled. Kiik et al. (2016) documented the behaviour of European mink kits and their mothers at Tallinn Zoo from the first days they left the nest box until separation. The results did not provide any indication of abnormal juvenile behaviour. Further research will be necessary to identify whether a detrimental effect occurs during early rearing, adolescence, or in a few cases even after initial reproductive success.

5. Conclusions

More than half of the captive males of this study were found to be unable or unwilling to express courtship and mating behaviour and did not sire any litters. One of the key elements of male courtship behaviour was the vocalisation “clucking”, which was found to be essential for a breeding attempt to end with copulation.

The behaviour of the males in response to chemical cues in experimental situations was similar to the mating behaviour when encountering an oestrous female. Female behavioural patterns did not relate to the outcome of the breeding attempt and gave no indication of their receptiveness for mating.

Conflict of interest

None.

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