Behavioral Ecology and Sociobiology (2019) 73: 137
https://doi.org/10.1007/s00265-019-2744-2

ORIGINAL ARTICLE

Overmarking behaviour of zebra males: no scent masking, but a group cohesion function across three species

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Received: 9 February 2019 / Revised: 19 August 2019 / Accepted: 21 August 2019 / Published online: 12 September 2019

Abstract

Among many hypotheses in the literature that explain overmarking in mammals, most studies favour a sexual selection hypothesis. However, results in the literature are conflicting. In this study, we tested two hypotheses that could explain overmarking by males: (i) as a part of sexual selection, more specifically to mask scent of receptive females and (ii) as a form of communication serving to aid group cohesion. We observed each of the three zebra species in eight different herds at four zoos. In total, we recorded 1395 eliminations (760 defecations, 635 urinations) performed by 78 individuals including 8 stallions. Stallions investigated 248 eliminations and overmarked 124. The rate of overmarking by stallions was higher than those of all other sex and age categories. Stallions of all species overmarked all age and sex categories, except Grévy's zebra stallions did not overmark foal eliminations. In contrast to our first hypothesis, when attracted to the elimination, stallions overmarked non-oestrus females more often than oestrus ones. Thus, our results did not support the hypothesis that overmarking by males has a sexual selection function, but it could be explained by the group cohesion hypothesis. Based on our results, it seems that overmarking by equid males plays a greater role in intra-specific communication than in intra-sexual competition. In addition, this behaviour might play different roles in different species based on their social organisation.

Significance statement

Overmarking is ubiquitous among terrestrial mammals; however, the function of this behaviour has not been fully explained. In addition, previous studies came to differing conclusions. In this study, we tested two hypotheses possibly explaining overmarking by adult males in three equid species. We found that adult males overmarked at a much higher rate than individuals of all other sex and age categories. In contrast to many other studies, our results did not support the hypothesis that overmarking by males has a sexual selection function, but it could be explained by a group cohesion hypothesis.

Keywords Competition · Defecation · *Equus* · Marking behaviour · Olfactory communication · Scent marking

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Communicated by K. E. Ruckstuhl

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00265-019-2744-2) contains supplementary material, which is available to authorized users.

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Introduction

Overmarking, when one individual places a scent mark directly over that of another individual (Johnston et al. 1994), is a widespread but still poorly understood phenomenon in mammals (Johnson 1973; Brown and Macdonald 1985; Brashares and Arcese 1999; Jordan et al. 2011a). Out of ten hypotheses reported in the literature (Table 1), most empirical support was given to the competition (Ferkin and Pierce 2007; Jordan 2007; Jordan et al. 2011a), territorial marking (Brashares and Arcese 1999), mate-attraction (Heymann 2006), and mate-guarding (Jordan et al. 2011b) hypotheses. Thus, in mammalian males, overmarking is often explained as a part of sexual selection in terms of intermale competition. This competitive hypothesis can have the proximate or ultimate function of the following: inciting competition (Hurst 1990a; Johnston et al. 1995; Jordan et al. 2011b), reproductive suppression (Snowdon et al. 1993), or maintenance of social hierarchy (Rich and Hurst 1998, 1999). In this context, it is interesting that although mammalian scent glands, secretions, and overmarking behaviour are commonly sexually dimorphic, outside rodents (Hurst 1990a, b; Gosling and Roberts 2001; Johnston 2008; Müller-Schwarze 2006), these traits have received little attention compared to avian plumage and mammalian weaponry (Jordan et al. 2011b). Owing to the costs of repeatedly monitoring and covering the scent marks of conspecifics, overmarking may provide an honest indication of a male's resource-holding potential. This perhaps explains why female rodents exposed to experimental overmarks subsequently prefer to associate with males whose scent mark was on top (Johnston et al. 1994, 1995).

Equids represent a suitable group for testing hypotheses explaining overmarking behaviour because they possess no specific glands so use only urine and faeces for overmarking (Moehlman 1985), and their rate of overmarking compared to most other mammalian families is high (Brown and Macdonald 1985; Moehlman 1985; Tučková et al. 2018). Since the volume of faeces used for overmarking is smaller than that in regular elimination, a specific location is chosen, and the behaviour used is often ritualised, it is clear that overmarking by faeces is intentional behaviour in equids (Tyler 1972).

Most research on equid overmarking behaviour was aimed at stallions (for a review see Moehlman 1985), but results of these studies are conflicting. Earlier studies concluded that overmarking by equid stallions in territorial species (Grévy's zebra *Equus grevyi*, African wild ass *E. africanus*, and Asiatic wild ass *E. hemionus*) serves as territory markers (Klingel 1975; Moehlman 1985; Kimura 2000), whereas in harem forming species (horses *E. ferus*, mountain zebra *E. zebra*, and plains zebra *E. quagga*), overmarking was considered a rudiment of territoriality (Tyler 1972; Penzhorn 1984). Later,

Table 1 The list of hypotheses explaining overmarking behaviour in mammals

Name of hypothesis	Taxa where supporting evidence was reported	Reference
Competitive advantage	Carnivores, primates, rodents, *Macrotis lagotis*, *Mazama gouazoubira*, *Gazella arabica*	Johnson and Johnson (1983); Hurst (1990a); Johnston et al. (1994, 1995); Kohli and Ferkin (1999); Palagi et al. (2004); Ferkin et al. (2004); Lewis (2005); Jordan (2007); Black-Decima and Santana (2011); Jordan et al. (2011a, b); Wronski et al. (2013)
Bulletin-board	*Microtus ochrogaster*, *Callithrix jacchus*, *Saguinus labiatus*	Lazaro-Perea et al. (1999); Smith and Gordon (2002); Wolff et al. (2002)
Territory marking	Carnivores, rodents, *Oreaotragus oreatragus*, *Ourebia ourebi*	Brashares and Arcese (1999); Gosling and Roberts (2001); Sillero-Zubiri and Macdonald (1998); Roberts and Dunbar (2000); Drea et al. (2002); Wolff et al. (2002)
Mate attraction	Carnivores, primates, rodents	Heymann (1998, 2006); Kappeler (1998); Hurst (1990b); Woodward et al. (2000); Drea et al. (2002); Ferkin et al. (2004)
Mate-guarding	Carnivores, primates, ungulates, *Microtus ochrogaster*	Moodie and Byers (1989); Brashares and Arcese (1999); Woodward et al. (1999, 2000); Drea et al. (2002); Ferkin et al. (2004); Palagi et al. (2004)
Food detection	*Mellivora capensis*, *Lutra lutra*	Begg et al. (2003); Kruuk (1995)
Group cohesion	*Callithrix jacchus*, *Canis simensis*	Sillero-Zubiri and Macdonald (1998); Lazaro-Perea et al. (1999)
Strengthening of social bonds	*Equus africanus*, *E. grevyi*, *E. quagga*, *E. zebra*	Tučková et al. (2018); Pluháček et al. (2019)
No function	*Microtus ochrogaster*, *Lutra lutra*	Thomas (2002); Rostain et al. (2004)
Multifunctional explanation		Ferkin and Pierce (2007)

the most accepted explanation was intra-male competition aiming to mask the scent of receptive (oestrous or non-pregnant) females, thus so-called concealment hypothesis (Turner et al. 1981; Moodie and Byers 1989). This hypothesis was supported in many ungulates where males overmark female eliminations (Barrette 1977; Dubost and Feer 1981; Moodie and Byers 1989; Muller-Schwarze 2006), including equids (Turner 1981; Boyd and Kasman 1986; Kimura 2000; Jezierski et al. 2015). Nevertheless, two objections to this hypothesis were raised in a seasonally reproductive species (horse). First, males overmarked females year-round, even when females were not receptive (Tyler 1972; King and Gurnell 2007); second, males do not overmark all female eliminations (Salter and Hudson 1982; King and Gurnell 2007). Therefore, some authors modify this hypothesis to a consort hypothesis (King and Gurnell 2007): overmarking serves to provide information to all other individuals that the stallion is associated with the respective mare.

Since males of harem dwelling species were observed to also overmark eliminations of subadults and foals (Feist and McCullough 1976; Penzhorn 1984), the other possible explanation of overmarking by stallions is group cohesion (Ödberg 1971; Lazaro-Perea et al. 1999). This hypothesis has received little attention despite some indirect support: females overmark more often in a stallion's absence than in his presence (Tyler 1972; Tučková et al. 2018). Since the stallion plays a prominent role in regulation of group size and supports group stability in harem dwelling species (Klingel 1972; Penzhorn 1984; Schilder 1992; Linklater et al. 1999), his overmarking rate should be higher than that of other herdmates.

In this study, we tested two hypotheses explaining overmarking behaviour performed by stallions of three captive zebra species: (i) masking the scent of receptive females and (ii) group cohesion function. If overmarking serves mainly to mask the scent of receptive females, we predicted that stallions should preferentially overmark faeces and urine of mares in oestrus and/or non-pregnant mares. If marking behaviour has a group cohesion function, then adult stallions should overmark all members of the herd non-discriminatively and at a higher rate than other age and sex categories. In addition, we also examined possible effects of other factors on various aspects of overmarking behaviour by stallions.

Material and methods

Animals

We observed three zebra species (Grévy's zebra, plains zebra, and mountain zebra) in four zoos (Brno, Dvůr Králové, Liberec, and Ústí nad Labem) in Czechia. In total, our observations involved 78 individuals including 8 adult males (stallions) in 8 herds (two herds of Grévy's, two of mountain zebra, and four of plains zebra; Table 2). Each herd contained one adult stallion. Herd size varied from 4 to 18 individuals. All herds were observed in outdoor enclosures (800 to 2800 m^2) that were mostly covered by gravel with some patches of grass. Food (grass or hay in feeders) and water were available ad libitum for each herd. Each enclosure was cleaned once a day in the morning before the start of the observation; thus, all faecal matter was removed daily.

Zebras were observed in 2010 from 24 June to 25 October and in 2011 from 1 June to 18 August. We performed two observation sessions each day: one in the morning (between 0800 and 1100 h) and other in the afternoon (1400 to 1700 h); no herd was observed twice in a day. Each herd in each season was observed five times a week during two non-subsequent weeks per season, resulting in ten observation sessions per herd per season. In total, we carried out 300 h of observation (100 sessions over 79 days). Most observations (> 95%) were performed by one observer (VT), with the remainder by one

Table 2 Sample size of herds of three zebra species observed in 2010 and 2011 in four zoos in Czechia

Species	Zoo and herd number	Number of all individuals observed in 2010 (stallions, mares-mares in oestrus, subadults, foals)	Number of all individuals observed in 2011 (stallions, mares-mares in oestrus, subadults, foals)
E. grevyi	Brno	5 (1, 3-2, 0, 1)	No stallion*
	Dvůr Králové	16 (1, 11-4, 3, 1)	No stallion
E. quagga	Brno	4 (1, 2-0, 1, 0)	6 (1, 2-0, 2, 1)
	Dvůr Králové 1	11 (1, 4-2, 3, 3)	No stallion
	Dvůr Králové 2	11 (1, 7-2, 0, 3)	No stallion
	Liberec	8 (1, 6-1, 0, 1)	7 (1, 6-1, 0, 0)
E. zebra	Dvůr Králové	14 (1, 7-3, 0, 6)	No stallion
	Ústí nad Labem	9 (1, 7-3, 1, 0)	No stallion

*Data from herds where no stallion was present were not used in this study other (JP). It was not possible to record data blind because our study involved focal animals in the field.

In each observation session, we recorded data concerning all observable eliminative events using the ad libitum sampling method (Altmann 1974). We used the same definition of marking as described by other researchers (Turner et al. 1981; Kimura 2000, 2001). Thus, the individual performing marking had to (i) deliberately move to the site of elimination (most cases of overmarking were initiated within 1 min, as defined by Turner et al. 1981), (ii) perform sniffing either before or after the marking, and (iii) defecate or urinate directly on the elimination (Turner et al. 1981; Kimura 2000). A detailed description of our methods including age category definitions can be found in Tučková et al. (2018).

Statistics

All data were analysed using the SAS System, Version 9.4 (SAS Institute, Inc. Cary, NC). To assess the impact of tested factors on marking behaviour of stallions, we applied an analysis of categorical repeated measurements based on the generalized estimating equation approach (Liang and Zeger 1986) using the logistic regression model (GENMOD procedure in SAS, distribution = binomial, link function = logit). The GENMOD procedure was designed to test the probability that the elimination was used for marking behaviour (i.e. for covering an elimination of another individual) (model A) and that the elimination was overmarked by a stallion (models B and C). Whereas all eliminations were analysed in model (B), only those where the stallion showed any reaction (at least sniffing) were used in model (C). We extracted data of adult females only to add a further model (C1) to examine eliminations of adult mares that were overmarked and/or investigated by the stallion.

The explanatory variables entered for each model and those that remained as significant in the final models are summarised in Table 3. We defined the “eliminator” as the initial animal urinating/defecating (i.e., providing the stimulus that was overmarked). For definitions of age category of eliminator (foal/subadult/adult), see Tučková et al. (2018). Female equids clearly demonstrate when they are in oestrus. This was determined by the observer based on the definition reported by Crowell-Davis (2007).

In all models, repeated measures on the same individuals were handled with each individual performing elimination (models A, B) or individual stallion performing overmarking (model C) entering the model as a subject in the repeated statement. We started with the full model including all of the fixed effects and sequentially dropped those effects which were not significant. Only factors that were statistically significant are referenced in the results section.

Results

Eliminations

In total, we recorded 1635 eliminations (868 defecations, 767 urinations) performed by 78 individuals. The eight individual stallions were responsible for 150 defecations and 167 urinations.

Overmarking behaviour of stallions

Stallions reacted to 106 defecations (15%, $n = 718$) and 142 urinations (24%, $n = 600$). They over-marked 56 (53%) defecations and 68 (48%) urinations (Table 4). Urine was overmarked by urination and faeces by defecation more often than vice versa ($\chi^2 = 4.81$; df = 1; $p = 0.0283$; $n = 124$; model was corrected for repeated measurements of overmarking by the same individual stallion $n = 8$). Stallions overmarked faeces by urine on only two instances when adult mares defecated and used urine over faeces overmarking two foals.

All but one of the stallions overmarked with urine as well as faeces. Before overmarking, stallions of all three species sniffed and flehmened in reaction to eliminations of foals, subadults and mares (Table 5). Stallions of Grevy’s and plains zebra both sniffed and flehmened after overmarking only on mare or subadult male eliminations. Mountain zebra stallions sniffed after overmarking mares or foals, but were never observed to flehmen after overmarking.

Stallions overmarked 45 out of 70 individuals at least once, including an individual of each age category at least once, with the exception of Grévy’s zebra where no foal ($n = 2$) was overmarked by the stallion (Tables 4 and 6). Overmarking including sniffing and flehmen was performed by stallions when mares were both pregnant, in oestrus or lactating, as well as when non-pregnant, in anoestrus, and non-lactating. Moreover, we recorded no case of flehmen by a stallion after he overmarked eliminations of mares in oestrus.

Who did most of the overmarking? (Model A)

The overall rate of overmarking was affected by the sex and age category of the eliminator ($\chi^2 = 15.48$; df = 5; $p = 0.0085$; Fig. 1) and the type of elimination ($\chi^2 = 7.08$; df = 1; $p = 0.0078$) and tended to be affected by species ($\chi^2 = 5.97$; df = 2; $p = 0.0506$). Rate of marking by stallions was higher than that of all other categories (Fig. 1). Across age and sex groups, urine was used for overmarking more frequently (17.5%, $n = 747$) than faeces (10.1%, $n = 868$). Grevy’s zebra individuals overmarked more often than mountain zebra individuals ($z = 2.37$; df = 1; $p = 0.0179$). Nevertheless, when only stallions were compared, the rate of marking did not differ among species. When we tested overmarking by urination and

Table 3 Overview of analyses including all variables. Those variables which remained in the final model and tended to be significant ($P < 0.1$) are in uppercase, with significance ($P < 0.05$) indicated by italic type. × indicates interactions of two variables

Analysis	A	B	C	C1
Dependent variable	The elimination was used for overmarking i.e. for covering an elimination of another individual	The elimination was overmarked by a stallion	The elimination was overmarked by the stallion (only cases when stallion examined elimination)	The elimination was overmarked by the stallion (only cases when stallion examined elimination of adult mares)
Independent variables				
Type of elimination (urination/defecation)	*X*	*X*	x	x
Sex and sex category of eliminator	*X*	x	X	x
Species	*X*	x	x	x
Zoo	x	x	x	x
Year (2010/2011)	x	x	x	x
Herd size (4–18)	x	*X*	x	x
The number of mares in the herd (2–11)[1]	x	*X*	x	x
Type of surface (concrete/grass/gravel/sand/soil)	x	x	x	X
Outside temperature	x	x	x	x
Occurrence of rain (yes/no)	x	x	x	x
Edge of enclosure (yes /no)	x	x	x	x
Pregnancy of eliminator (yes/no)		x		x
Lactating status of eliminator (yes/no)		x		x
Oestrus status of eliminator (yes/no)		x		*X*
Pre-oestrus status of eliminator (yes/no)[2]		x	x	*x*
Behaviour of eliminator preceding the elimination (walking/standing/feeding/other)		x	x	x
Breaking of movement of the eliminator (walking/breaking/standing)		*X*	x	x
Age of the stallion		x	x	x
Flehmen performance by stallion (yes/no)		x	*X*	x
Sex and age category of the eliminator × type of elimination	x	x	x	x
Herd size × type of elimination.	x	x	x	x

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[1] Since the variables "herd size" and "number of adult mares" were intercorrelated, we performed separate models with each of them. We left the better model (herd size) in the results based on QIC fit criteria

[2] Pre-oestrus status was defined as 1 week before we observed oestrus. This period might be detectable by conspecifics but is not visually apparent to humans

Table 4 Types of overmarking behaviour performed by stallions

Timing of behaviour	Behaviour	Faeces	Urine
Before	Sniff	106	142
	Flehmen	0	67
	Pawing	1	3
Marking	Defecation	66	2
	Urination	2	54
After	Sniff	6	17
	Flehmen	0	19
	Pawing	0	0
Total number of eliminations		718	600

defecation separately, the results were similar (Supplementary Tables S1, S2, S3).

Who was overmarked by stallions? (Model B)

When considering all eliminations, we found that overmarking by stallions was affected by the type of elimination ($\chi^2 = 4.20$; df = 1; $p = 0.0403$), number of animals within the herd ($\chi^2 = 8.51$; df = 1; $p = 0.0035$), and breaking of movement due to elimination by the eliminator (i.e., the individual stopped moving to urinate or defecate; $\chi^2 = 10.82$; df = 2; $p = 0.0045$). Stallions over-marked urine (11.0%, $n = 600$) more often than faeces (7.8%, $n = 718$), and they over-marked more often when the eliminator was standing (8.7%, $n = 767$) than when it was walking (2.8%, $n = 138$; $z = 2.57$; $p = 0.0102$). The probability of overmarking by stallions decreased with an increasing number of animals within the herd (Fig. 2). This remained true when we excluded foals and subadults to examine the number of mares in the herd ($\chi^2 = 7.81$; df = 1; $p = 0.0052$). However, the overall generalised estimated equation (GEE) fit criteria of mares only were worse than for the whole herd (QIC = 788.1127 vs QIC = 782.4404; QIC = quasilikelihood under the Independence model Criterion, Pan (2001)). None of the other tested factors were significant (Table 3). When we tested overmarking by urination and defecation separately, the results were very similar (Supplementary Tables S1 and S2).

In addition, the number of individuals overmarked at least once by a stallion correlated with herd size (Pearson $r = 0.88$, $n = 10$, $P < 0.001$). Similarly, the number of overmarked mares correlated with number of mares in the herd (Pearson $r = 0.77$, $n = 10$, $P = 0.0089$).

When did stallions overmark an elimination? (Model C)

When considering only cases where stallions reacted to an elimination (sniffing, $n = 247$), we found that the stallion's decision to overmark ($n = 124$) was associated with flehmen. Overmarking followed a flehmen response to the elimination more often (67.2%, $n = 67$) than when it did not (43.3%, $n = 180$; $\chi^2 = 4.06$; df = 1; $p = 0.0440$). The stallion performed flehmen in at least one case for each age category (adult, subadult, foal), except for subadults in Grévy's and mountain zebra. The interaction between sex and age of the eliminator tended to be significant ($\chi^2 = 3.68$; df = 1; $p = 0.0550$); stallions tended to overmark males (i.e. male foals and subadults) more often than females.

When considering only reactions of stallions towards adult mare eliminations ($n = 197$; model C1), overmarking was affected by oestrus state ($\chi^2 = 3.93$; df = 1; $p = 0.0473$): stallions overmarked mares in anoestrus more often (54.7%, $n = 49$) than those in oestrus (30.6%, $n = 148$). No other factor including pre-oestrus, lactation, or pregnancy was significant.

Table 5 Number of individuals of various age and sex categories which were overmarked at least once by the stallion in three captive zebra species

Species	Age and sex category of eliminator	Number of all individuals that were overmarked by stallion at least once	Number of all individuals
E. grevyi	Adult female	11 (79%)	14
	Subadult female	1 (50%)	2
	Foal female	0 (0%)	2
E. quagga	Adult female	15 (83%)	18
	Subadult female	1 (33%)	3
	Subadult male	2 (100%)	2
	Foal female	2 (50%)	4
	Foal male	1 (25%)	4
E. zebra	Adult female	6 (43%)	14
	Subadult female	1 (100%)	1
	Foal female	4 (80%)	5
	Foal male	1 (100%)	1
Total		45 (64%)	70

Table 6 Number of defecations and urinations overmarked by stallions of three zebra species in four zoos in Czechia in 2010 and 2011

Species	Zoo and herd number	Number of defecations by all individuals except stallion/number overmarked by stallion (percentage)		Number of urinations by all individuals except stallion/number overmarked by stallion (percentage)	
		2010	2011	2010	2011
E. grevyi	Brno	31/3 (10%)	NA	11/3 (27%)	NA
	Dvůr Králové	125/3 (2%)	NA	125/12 (10%)	NA
E. quagga	Brno	29/7 (4%)	43/3 (7%)	15/7 (46%)	31/5 (16%)
	Dvůr Králové 1	9/2 (22%)	NA	15/2 (13%)	NA
	Dvůr Králové 2	88/0 (0%)	NA	61/9 (15%)	NA
	Liberec	74/3 (4%)	64/20 (31%)	49/5 (10%)	83/14 (16%)
E. zebra	Dvůr Králové	148/6 (4%)	NA	107/6 (6%)	NA
	Ústí nad Labem	107/9 (8%)	NA	103/3 (3%)	NA

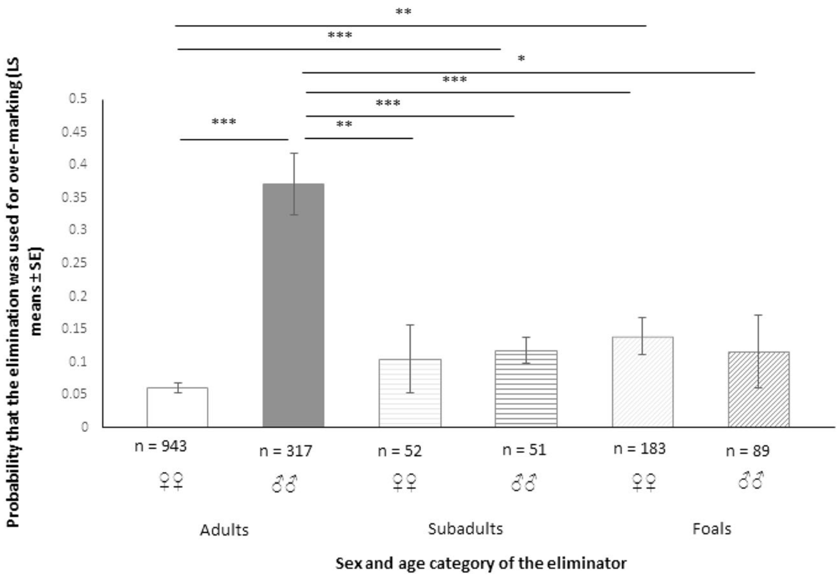
NA not applicable

Discussion

Similar to previous studies on equids and other mammals, we found that adult males overmarked more often than other age and sex categories (Tyler 1972; Barette 1977; Muller-Schwarze 2006; King and Gurnell 2007; Wronski et al. 2013). Stallions overmarked individuals of each sex and age category (except foals in Grévy's zebra), with no preference for non-pregnant and oestrous mares. Interestingly, we found that after inspecting an elimination, stallions overmarked mares in anoestrus more often than those in oestrus. It could be argued that the more important time to detect location in the reproductive cycle might be in the pre-oestrous period. However, we did not find higher overmarking rate by stallion in the pre-oestrus period. Thus, our results did not confirm the hypothesis that overmarking should serve to mask the scent of receptive females. This is surprising as the hypothesis of overmarking as a form of direct sexual competition was favoured in many studies on mammals (Ralls 1971; Gosling and Roberts 2001; Jordan et al. 2011a), including equids (Feist and McCullough 1976; Turner 1981; Boyd and Kasman 1986; Kimura 2000, 2001; Jezierski et al. 2015). Other equid studies confirm our results as they showed no evidence for the scent masking hypothesis (Tyler 1972; King and Gurnell 2007).

On the other hand, non-discriminative overmarking by stallions is in line with a group cohesion hypothesis. During our study the stallions overmarked 64% of their herdmates at least once. When considering only adult females (the core members of a group), this rises to 70% of individuals available to overmark. The fact that not all individuals were overmarked may reflect preferred associations between certain individuals, equating to individuals who would remain socially bonded in a wild group. In harem-forming equid species (e.g. plains and mountains zebras), the male is largely responsible for group

Fig. 1 Rate of eliminations used for over-marking according to sex and age category across three zebra species in captivity (significance levels indicated by the following: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)



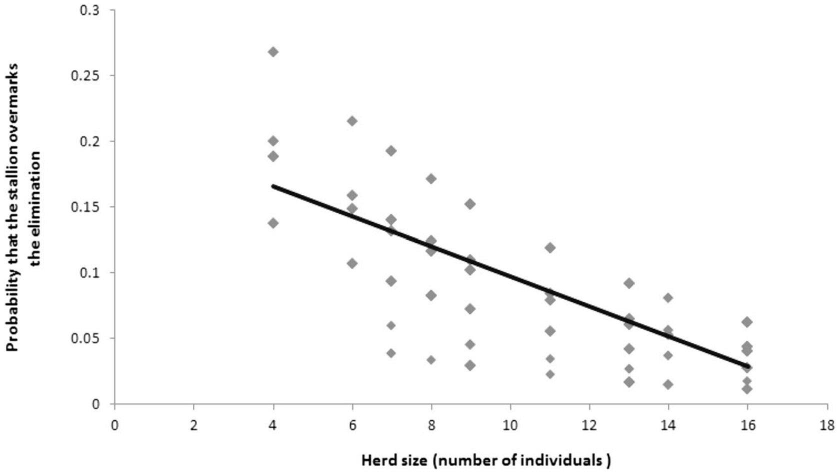


Fig. 2 Probability that the elimination was overmarked by a stallion according to herd size in terms of number of individuals in three captive zebra species

cohesion (Klingel 1972; Penzhorn 1979). This fits well with our finding that adult males overmarked more often than other age and sex categories did, as found in other mammals (Hurst 1990a). To further favour this hypothesis, overmarking has been observed in non-reproductive equid groups (Tučková et al. 2018). Overmarking among group members of bachelor herds of plains and mountain zebra has been reported (Klingel 1967; Penzhorn 1984), and in a previous study, we found that adult equid females overmarked at a higher rate when the stallion was absent than when he was present (Tučková et al. 2018). The group-cohesion hypothesis might be well applied to harem dwelling species, but it does not explain stallion overmarking in Grévy's zebra, which form only short-term associations (Sundaresan et al. 2007). Thus, stallion overmarking in this species could be related to alternative explanations like the social bond hypothesis (Tučková et al. 2018) or be a non-adaptive rudimentary behaviour. Male rodents overmark other males often to gain competitive advantage over them (Hurst 1990a; Müller-Schwarze 2006; Johnston 2008). In our study, only one adult male was present in each group, thus, we cannot explore this here.

In rodents, the rate and form of overmarking by males is affected by social organisation of species (Ferkin et al. 2004; Ferkin and Pierce 2007; Johnston 2008). Although wild equid species form two different types of social organisation (Klingel 1975), our sample size was too small to test any interspecific differences. The only interspecific difference we found was the absence of overmarking of Grévy's zebra foals by stallions, similar to what was observed in the wild (Klingel 1974; Kimura 2000). This might be explained by different social organisation of the respective species. Whereas mountain and plains zebra mares together with their foals form stable groups including a stallion (Klingel 1972; Penzhorn 1979), Grévy's zebra females form only loose associations (Klingel 1974; Rubenstein 1989; Sundaresan et al. 2007), with breeding stallions forming short-term associations with females (Ginsberg 1989). If overmarking serves to assist group cohesion, then the differences in social organisation might explain the observed absence of overmarking of foals by stallions in Grévy's zebra and the relatively high rate of overmarking of foals by stallions in mountain zebra, where strong stallion-foal bonds were recorded (Penzhorn 1984). However, it should be noted that there were only 2 Grévy's zebra foals in the groups we observed, so this could be due to lack of opportunity rather than a tendency to not overmark foals.

We found that stallions overmarked more often when they checked the elimination by flehmen. This supports the importance of flehmen for recognition of individual odours (Marinier et al. 1988; Stahlbaum and Houpt 1989), but not only associated with reproductive behaviour as suggested previously (Hart 1987; but see Stahlbaum and Houpt 1989): stallions also flehmened after exploring eliminations of herdmates other than adult mares.

The rate of overmarking by stallions decreased with increasing herd size. Since equids do not possess any specific glands and overmarking is performed by faeces and urine, this finding might reflect physiological limits for overmarking even when a small volume is used (Tyler 1972). On the other hand, the number of individuals overmarked by the stallion at least once correlated with the herd size. This result showing non-discriminative overmarking is supportive of a group cohesion hypothesis. It should be noted that the effect of herd size on a stallion's overmarking behaviour might also vary according to the social organisation of the species. Unfortunately, we were not able to test this due to the limited number of herds we observed.

Our results come from a captive situation where herd size varied. Nevertheless, the herd size of animals we observed was similar to data reported from the wild (Grévy's zebra—1-17: Sundaresan et al. 2007; Parker et al. 2011; plains zebra—2-16 Klingel 2013; mountain zebra—2-13: Penzhorn 2013). The mountain zebra herds we observed included more adult mares than in the wild (Penzhorn 1984, 2013), which might explain why stallions overmarked only some of them: they could prefer formation of smaller herds in line with group cohesion hypothesis. Similarly, the reproduction and breeding period of each species in the zoos where we conducted research (see Dobroruka et al. 1987; Pluháček et al. 2006) corresponded to those reported from the wild, where reproduction is aseasonal with several peaks reflecting the wet season (Klingel 2013; Moehlman et al. 2013; Penzhorn 2013). Interbirth intervals averaged around 2 years for all these species in the wild (Klingel 2013; Moehlman et al. 2013) as well as in the zoos in our study (Dobroruka et al. 1987; Pluháček et al. 2006).

In our previous studies, we found that overmarking in mares (Tučková et al. 2018) and foals (Pluháček et al. 2019) was associated with affiliative relationships (social bonds) within the herd. Unfortunately, we had no data to test this hypothesis for stallions, but future research on this topic might be important to clarify stallion overmarking behaviour. Similarly, future studies on bachelor groups may be useful to examine whether overmarking is related to dominance behaviour in male equids. Consistent with other studies, we found that stallions tended to overmark older individuals of both sexes, especially males (Zharkikh and Andersen 2009; Krueger and Flauger 2011). Age is positively associated with dominance in many mammals, including equids (Pluháček et al. 2006; Šárová et al. 2013). It is also common for dominant individuals to overmark more often than submissive (Ralls 1971; Müller-Schwarze 2006), and overmarking down the hierarchy has been observed in feral and Przewalski's horses (Moehlman 1985; Zharkikh and Andersen 2009). In our previous study, we found no relationship between overmarking and dominance hierarchy among equid mares (Tučková et al. 2018), but as only one stallion was present in each group, we could not determine whether overmarking was related to dominance hierarchy among males. However, it should be noted that the meaning of overmarking might differ between males and females (Wronski et al. 2013).

In conclusion, it seems that overmarking by equid males is more a form of communication than related to sexual selection. This behaviour might play different roles in harem-dwelling species and territorial species. Whereas in harem-dwelling species overmarking by stallions serves to aid group cohesion, the meaning of overmarking by stallions remains unclear in territorial species. While our data favour the social cohesion hypothesis, a larger data set may provide evidence supporting other hypotheses, such as strengthening social bonds or expression of dominance, for the function of male overmarking in equids. In ecology, there is rarely only one answer for a question, so support for one hypothesis does not exclude support for the social cohesion hypothesis as the function may be related to context.

Acknowledgments The authors gratefully acknowledge the assistance of the Zoological Garden staff at Dvůr Králové, Brno, Liberec, Ostrava, and Ústí nad Labem, in particular Luděk Čulík, Markéta Čulíková, Aleš Kopecký, Jiří Soumar, Miroslava Kubelková, Miroslava Doležalová, Pavel Moucha, Radek Hlávka, Lubomír Melichar, Jiří Vítek, Petra Padalíková, Pavel Král, Jaroslav Novák, Rostislav Střižík, Eva Zajoncová, and Lenka Málcová. We thank Radka Šárová, Luděk Bartoš, and Jitka Bartošová for their helpful comments on the earlier draft of the manuscript. The manuscript was highly improved by comments of four anonymous reviewers.

Funding information This work was supported by the Ministry of Agriculture of the Czech Republic (MZE-RO0719) and by grant no. 2011/008 of the Student Grant Agency of the University of South Bohemia.

Data availability The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and institutional guidelines for the care and use of animals were followed. This research was carried out in line with all legislation of European Union and the Czech Republic. We received all appropriate permissions from the zoo authorities where the observations were performed. As this was an observational study with no manipulation or disturbance of the animals no animal care and use protocols were required.

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