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Scent-marking behavior by female sloth bears during estrus

Yaduraj Khadpekar[1,3,4], John P. Whiteman[2], Barbara S. Durrant[2], Megan A. Owen[2], and Sant Prakash[1]

[1]*Department of Zoology, Dayalbagh Educational Institute, Dayalbagh, Agra, Uttar Pradesh, 282005, India*
[2]*Institute for Conservation Research, San Diego Zoo Global, 15600 San Pasqual Valley Road, Escondido, CA 92027, USA*
[3]*Wildlife SOS, D-210, Defence Colony, New Delhi 110024, India*

Abstract: The sloth bear (*Melursus ursinus*) is one of the least studied bears. Important aspects of sloth bear biology and ecology, such as reproductive physiology and behavior, are largely unknown. Increased scent-marking by anogenital rubbing during breeding season has been recorded in other bear species. We studied the genital rubbing behavior of 37 captive female sloth bears (2–18 yr of age) at the Agra Bear Rescue Facility, India, for 4 breeding seasons over a period of 3.5 years (1 Jun 2015 to 31 Dec 2018). Data on changes in vulva visibility and presence of genital rubbing behavior were collected daily during the breeding period and twice per week for rest of the year, throughout the study period. Vulva visibility was scored as 0 (not visible), 1 (slightly visible), and 2 (fully visible), and a female was considered to be in estrus if the vulva was slightly or fully visible. Presence of genital rubbing was recorded as 1 and its absence as 0. Occurrence of genital rubbing coincided with estrus, as defined by vulva visibility scores. Statistical analysis indicated that female age and the number of males with physical proximity (i.e., in the same enclosure) were significantly correlated with the occurrence of genital rubbing behavior. The number of females in physical proximity and the number of females in the vicinity without physical proximity (i.e., not in the same enclosure but sharing a fence) did not significantly affect this behavior. The results of our study suggest that the genital rubbing behavior by female sloth bears in estrus is likely a form of scent-marking, serving a communicative function, and could be influenced by male presence. This behavior may be a key factor in attracting a mate during the breeding season in the wild.

Key words: chemical communication, estrus, genital rubbing, keeper check sheets, *Melursus ursinus,* reproductive behavior, sloth bear, vulva scores, zoo research

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For animals living under human care, long-term data collection from a relatively large number of individuals may be accomplished using systematically collected keeper feedback. Indeed, feedback from animal keepers has been used effectively in studies of reproduction in black rhinoceros (*Diceros bicornis;* Carlstead et al. 1999), cheetah (*Acinonyx jubatus;* Wielebnowski 1999), Asian elephant (*Elephas maximus;* Duer et al. 2016), and giant panda (*Ailuropoda melanoleuca;* Kleiman et al. 1979). This approach has proven to be advantageous in rescue sanctuaries and conservation centers for bears. For example, Khadpekar et al. (2018) recently demonstrated the research benefits of utilizing the working knowledge of keepers regarding the behavioral dynamics of sloth bears (*Melursus ursinus*) in their charge, for acquiring valid and reliable behavior data on large numbers of bears; particularly when logistical constraints preclude the hiring of dedicated research staff.

The sloth bear is one of the 4 bear species found in India (Menon 2014). It is distributed throughout the Indian subcontinent, including Nepal and Sri Lanka. Recently, it was found to be extirpated from Bangladesh (Islam et al. 2013). Distribution of the remaining population is patchy and corresponds with forest cover (Garshelis et al. 1999, Yoganand et al. 2006). Although there are no reliable population estimates currently, habitat degradation, human–bear conflicts, and poaching are considered to be major threats likely causing population declines (Dharaiya et al. 2016).

Despite being a relatively common bear species in India, as well as in zoos worldwide, the sloth bear is

[4]e-mail: yaduraj.k24@gmail.com

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arguably one of the least studied and least understood ursids. Limited information is available on the basic aspects of sloth bear biology, such as diet, behavior (Joshi et al. 1995, 1997, 1999), and reproductive physiology (Puschmann et al. 1977). From the scant data available, sloth bears are thought to be seasonal breeders (Spady et al. 2007). Puschmann et al. (1977) described finding a free blastocyst in the uterus of a female sloth bear 4 months after mating, indicating the likelihood that sloth bears experience delayed implantation. However, to our knowledge, there are no detailed studies that describe reproduction and related behavior in this bear species.

It is important to understand how sloth bears signal their reproductive status because their patchy distribution and declining numbers in an increasingly human-dominated landscape may hinder the ability of each sex to locate the other for successful mating (Wilmers et al. 2013). A reduction in encounter rates caused by low population density—an "Allee effect"—can have population-level consequences (Courchamp et al. 1999, Stephens and Sutherland 1999). Scent-marking can be used by mammals for reproductive communication (Eisenberg and Kleiman 1972), and this may be especially important for solitary animals, such as sloth bears, for assessing the location and status of potential mates in advance of face-to-face encounters (Owen et al. 2014). For example, there are a number of studies on different species of primates that describe urogenital scent-marking, such as by rubbing the vulva on a branch by bringing it forward while sitting (Horwich 1983, Lazaro-Perea et al. 1999). Female giant pandas are known to scent-mark by squatting and rubbing the anogenital region on horizontal surfaces, or by rubbing it on vertical surfaces with a leg-lift posture (Kleiman et al. 1979). This method of scent communication is especially prominent during estrus (Bonney et al. 1982, Lindburg et al. 2001, McGeehan et al. 2002). Brown bears (*Ursus arctos*) and American black bears (*U. americanus*) are known to scent-mark by body rubbing on trees and by pede-marking (Green and Mattson 2003, Taylor et al. 2015). Andean bears (*Tremarctos ornatus*) have also been observed to scent-mark trees by body rubbing (Filipczyková et al. 2016). However, there is very limited information available on scent-marking by sloth bears. Laurie and Seidensticker (1977) observed sloth bears scraping tree trunks and rubbing their flanks on those trees. Recently, Swaminathan et al. (2017) reported pede-marking behavior by sloth bears in the wild.

In this study, we hypothesized that genital rubbing in female sloth bears is a form of scent-marking, used to convey information regarding estrous status to male conspecifics. We predicted that observed rubbing behavior would peak during estrus. We tested this expectation using keeper-collected behavioral data from 37 captive individuals over a period of 3.5 years.

Study site

The study was carried out at the Agra Bear Rescue Facility (ABRF) at Agra in the state of Uttar Pradesh, India (27°15′14.76″N 77°51′17.07″E). The ABRF is located inside the Soor Sarovar Bird Sanctuary and spans 2 sides of the Yamuna River. It houses sloth bears rescued from the 'dancing bear' trade (Seshamani and Satyanarayan 1997) and from human–bear conflicts. When we initiated the study, the facility housed 224 sloth bears. The bears were housed in 14 separate enclosures of different configurations, ranging in size from 2,100 to 11,800 m^2. All but one enclosure held a mixed sex group. Each enclosure in ABRF comprises a den and an open field attached to it with an electric fence boundary. The dens are enclosed cemented rooms used for feeding and other husbandry purposes. In general, the bears have free access to the dens and attached fields.

Methods

Study animals

We selected 37 female sloth bears ranging in age from 2 to 18 years at study initiation. Of these, 1 bear was <5 years, 5 bears were between 5 and 10 years, 12 bears were between 10 and 15 years, and 19 bears were >15 years of age. All these bears were rescued, so their ages were approximate, with the probable error of ± 6 months. We included ≥ 1 female from each of the 14 enclosures in the study. With 2 exceptions, all females shared their enclosure with ≥ 1 males. All males had been previously neutered and thus all females were unmated throughout the study period. All the study females in mixed-sex groups were housed with males that were >5 years old. One female that was housed alone at the start of the study was later moved to another enclosure, where she was housed with 2 3-year-old males.

Data collection

At the start of the study, we created a data-collection check sheet for keepers. The check sheet was designed to collect information on 10 physiological and behavioral parameters, including information on vulva visibility and the presence or absence of genital rubbing behavior (Fig. 1). Before beginning data collection, we trained the primary keepers from all enclosures on how to use the

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Bear Name_____

Date_____ Month_____ Year_____

Vulva is:	**Vulva colour:**	**Meals skipped:**	**Aggressiveness**
Not visible:	Grey:	1:	Less:
Slightly visible:	Pink:	2:	Normal:
Fully visible:	Red:	3:	More:
Mounting others:	**Mounting received:**	**Rubbing vulva:**	**Mating vocals:**
None:	None:	Yes:	Yes:
Once or twice:	Once or twice:	No:	No:
More than twice:	More than twice:		
Water play:	**Activity:**	**Notes:**	
None:	Less:		
Normal:	Normal:		
More:	More:		

Fig. 1. Keeper check sheet used for data collection on female sloth bear (*Melursus ursinus*) behavior in estrus at the Agra Bear Rescue Facility, India (1 Jun 2015 to 31 Dec 2018). Keepers marked the applicable category for each parameter on the day of data collection.

check sheets to collect data. Training included example images of bears to standardize observations. On the check sheets, vulva visibility observations were categorized as (a) not visible ('0'), (b) slightly visible ('1'), and (c) fully visible ('2'; Fig. 2). Genital rubbing was described as rubbing the vulvar region on the den floor, den walls, the ground in the open field, or other substrates (e.g., water trough, wooden logs, trees), and was categorized as present ('1') or absent ('0'). Keepers marked the applicable category for each parameter on the check sheet at the end of their duty time; thus, the data reflected the opportunistic observations during their husbandry duties between 0800 and 1700 hours. Data collection began on 1 June 2015, with the exception of one female that entered the study in May 2017, and continued until 31 December 2018. It was carried out either daily (Apr through Jul; the observed breeding season in sloth bears; unpublished observations from ABRF) or twice per week outside the breeding season (Aug through Mar). In the absence of the primary keeper of a study bear, the replacement keeper had been previously trained in data collection and could identify the bear. Females with a vulva visibility score of 1 or 2 were considered to be in estrus (Durrant et al. 2003). Keepers collected 19,433 bear-days of observations. Keepers carried out data collection as approved by the Institutional Animal Care and Use Committee of San Diego Zoo Global (permit 14-036).

Fig. 2. Photographs of (a) a sloth bear (*Melursus ursinus*) exhibiting vulva visibility during estrus. Visibility scoring system used for data analysis included (b) 0—not visible, (c) 1—slightly visible, and (d) 2—fully visible. Images are of the captive female sloth bears at the Agra Bear Rescue Facility, India (1 Jun 2015 to 31 Dec 2018).

Data analysis

We did all statistical analyses using Program R (R Core Team 2018). We visually examined the relationship between vulva visibility and genital rubbing behavior by plotting vulva visibility and rubbing behavior data along the timeline of the entire study period. We carried out 3 different analyses using mixed-effects binomial logistic regressions (Program R—package lme4; Bates et al. 2015) to evaluate the correlation of selected study factors with genital rubbing behavior by female bears. In the first analysis, presence or absence of genital rubbing behavior was the response variable. Predictors included a fixed

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effect of vulva visibility score on the given day, and a random effect of individual bear ID.

The second analysis addressed estrus duration and influence of proximity to other females and males. We used the number of days during which each bear exhibited genital rubbing out of the entire estrus length in each year as a response variable. Fixed effects predictors included (1) bear age, (2) total duration of estrus that year for that bear (as indicated by vulva visibility), (3) number of males in the same enclosure (i.e., with physical proximity), (4) number of females in the same enclosure, (5) number of males in the adjacent enclosure sharing a fence (i.e., with only auditory, visual, and olfactory proximity), and (6) number of females in the adjacent enclosure sharing a fence. We added individual bear ID and year of data collection as random effect predictors. Based on the results of this model and our expectation that the physical proximity with males would be the best predictor of genital rubbing, we also assessed a model with fewer predictors. The predictors in this model included the individual fixed effect of number of males in the same enclosure, and the random effects of individual bear ID and year of data collection. The response variable was the same as the previous model. We compared the model performance between these 2 models with Akaike's Information Criterion (AIC) scores.

The third analysis addressed the consequence of changes in female proximity to males. During the study period, 4 bears were moved to different enclosures for husbandry purposes. For 3 of those bears, this move substantially changed the number of males across the fence. The other bear that was initially housed alone was moved to another enclosure with 2 males. The third analysis was similar to the second analysis, but was carried out using only the data on these 4 bears. However, the data from 4 bears were not sufficient to include all the predictors in a single model; therefore, we used 2 different models to assess the individual fixed effects of (1) number of males in the same enclosure and (2) number of males in the adjacent enclosure sharing a fence, respectively. In each of these models, the response variable and the random effects were similar to the second analysis. Some of the bears were already in estrus at the start of the data collection in June 2015, so we excluded data for those bears in that year from all statistical analyses.

Results

During estrus, female bears exhibited genital rubbing on vertical surfaces such as den walls, den metal gates and tree barks in the field, as well as on the horizontal surfaces and substrates such as den floor, grass, wooden logs, and natural floor in the field. Female bears carried out genital rubbing on the vertical surfaces by backing up to them and rubbing the vulvar region on the surface a few times in horizontal pattern while standing. On horizontal surfaces, bears did this by squatting on the hind legs and rubbing the vulvar region by moving it back and forth a few times. In both cases, a wet patch was observed on the surface after the female had moved away. It is not clear whether it was genital secretion or urinary excretion.

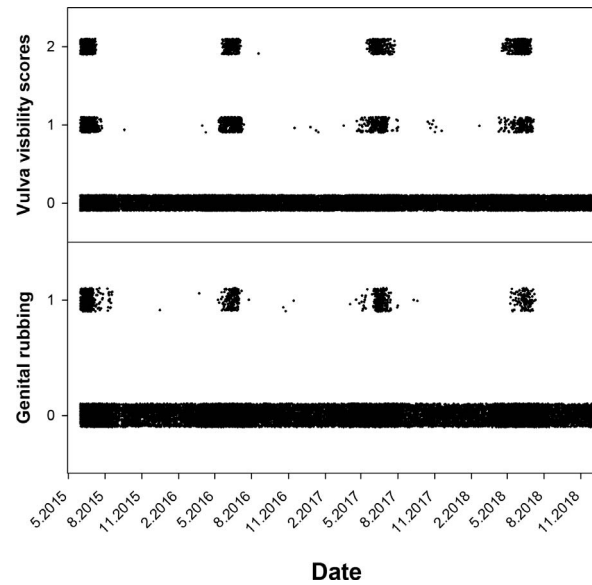

Fig. 3. Occurrence of high vulva visibility and genital rubbing behavior in female sloth bears (*Melursus ursinus*) during the study period. Vulva visibility was 0 (not visible), 1 (slightly visible), and 2 (fully visible). Presence of genital rubbing behavior was 1 (present) or 0 (absent). Each data point represents one day for one bear. Data points are slightly offset in X and Y space for visual clarity. The occurrence of genital rubbing behavior coincided with the period of high vulva visibility, used as an indicator of estrus. The data were collected over 3.5 years (1 Jun 2015 to 31 Dec 2018) from 37 captive female sloth bears at the Agra Bear Rescue Facility, India.

The occurrence of vulva visibility (scores 1 and 2) and genital rubbing (score 1) coincided temporally (Fig. 3). Vulva visibility scores of 1 and 2 both had a significant positive effect on genital rubbing behavior (Table 1). These results suggested that the onset of genital rubbing coincided with vulvar swelling. The results of the second statistical analysis are presented in Table 2. The age of the bear was observed to have a significant negative effect on the period for which genital rubbing was

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Table 1. Results of mixed-effects binomial logistic regression analysis with binomial response variable of presence (score of 1) and absence (score of 0) of genital rubbing of female sloth bears (*Melursus ursinus*). Fixed effects predictor was the ordered categories for vulva visibility as 0 (not visible), 1 (slightly visible), and 2 (fully visible). Individual bear ID was a random effects predictor. The data were collected over 3.5 years (1 Jun 2015 to 31 Dec 2018) from 37 captive female sloth bears at the Agra Bear Rescue Facility, India.

Predictor	Parameter estimate	SE	*z*	*P*
Vulva visibility score[a]				
1 (slightly visible)	3.575	0.137	26.15	<0.001
2 (fully visible)	5.228	0.131	39.81	<0.001

[a]Categorical variable: Score of 0 = reference category.

exhibited during estrus. Estrus duration did not have significant effect. The number of males in the same enclosure had a marginal positive significant effect. The number of females in the same enclosure and across a fence, and the number of males across a fence, were nonsignificant predictors. When the individual fixed effect of number of males in the same enclosure was evaluated, it was found to have a significant positive effect ($z = 1.96$, $P = 0.049$). The AIC scores of the 2 models used for this analysis were 670.6 and 667.6, respectively.

When we analyzed only the 4 bears that were moved to different enclosures, the number of males in the same enclosure was found to have a significant positive effect on genital rubbing behavior. The number of males across a fence had a marginal positive significant effect (Table 3).

Table 3. Results of mixed-effects binomial logistic regression analysis on the data from 4 sloth bears (*Melursus ursinus*) that were moved to a different enclosure during the study period. The response variable was the number of days on which each bear exhibited genital rubbing out of the entire estrus length in each year. Random effects predictors included individual bear ID and year of data collection. The data were collected over 3.5 years (1 Jun 2015 to 31 Dec 2018) from captive female sloth bears at the Agra Bear Rescue Facility, India.

Predictor	Parameter estimate	SE	*z*	*P*
No. of males in same enclosure[a]	3.238	1.165	2.78	0.005
No. of males across fence[b]	0.124	0.070	1.79	0.073

[a]The number of males in the same enclosure indicates the no. of males with whom the study female bears had physical proximity during estrus.

[b]The number of males across fence indicates the no. of males with whom the study female bears had only auditory, visual, and olfactory proximity (i.e., not in the same enclosure but sharing a fence) during estrus.

Discussion

We found that genital rubbing behavior in female sloth bears was positively associated with estrus, as indicated by vulvar swelling (i.e., visibility). The fact that female genital rubbing behavior was more likely to occur in the presence of males than females suggests that it is a form of scent-marking, playing a role in inter-sexual communication.

Scent communication has been documented in many species of ursids; however, the form of scent deposition

Table 2. Results of mixed-effects binomial logistic regression analysis with response variable of the number of days on which each female sloth bear (*Melursus ursinus*) exhibited genital rubbing out of the entire estrus length in each year. Random effects predictors included individual bear ID and year of data collection. The data were collected over 3.5 years (1 Jun 2015 to 31 Dec 2018) from 37 captive female sloth bears at the Agra Bear Rescue Facility, India.

Predictor	Parameter estimate	SE	*Z*	*P*
Age of the bear	−0.096	0.047	−2.03	0.043
Estrus duration	−0.013	0.009	−1.35	0.178
No. of males in same enclosure[a]	0.090	0.050	1.81	0.070
No. of females in same enclosure[a]	−0.024	0.056	−0.42	0.671
No. of males across fence[b]	0.014	0.051	0.28	0.780
No. of females across fence[b]	−0.040	0.041	−0.97	0.333

[a]The numbers of males and females in the same enclosure indicate the no. of bears of different sexes with whom the study female bears had physical proximity during estrus.

[b]The numbers of males and females across fence indicate the no. of bears from different sexes with whom the study female bears had only auditory, visual, and olfactory proximity (i.e., not in the same enclosure but sharing a fence) during estrus.

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varies between species, and the specific functional role of chemical communication has not been well-described for bear species other than the giant panda (Swaisgood et al. 2000). Available information on scent communication of the giant panda comes primarily from the long-term studies of captive individuals (Swaisgood et al. 2000, Lindburg et al. 2001, McGeehan et al. 2002). For the rest of the bear species, the few studies that exist come from wild bears and they primarily report scent-marking by males, not females. Green and Mattson (2003) observed that body rubbing (mostly the dorsal region) on trees by brown bears in the Yellowstone region (USA) peaked during the mating season. They did not specify whether it was the case for both males and females. However, Clapham et al. (2012) noticed that free-ranging adult male brown bears at Glendale Cove, British Columbia, Canada, scent-marked with greater frequency during the breeding season, although the adult females did not appear to increase scent-marking to advertise their estrous state. The males also appeared to use scent-marking for communicating dominance to conspecifics. Brown bears in general tended to choose conspicuous trees on frequently visited trails to scent-mark, and the scent-marking behavior and motor patterns changed with increasing age (Clapham et al. 2013, 2014). Taylor et al. (2015) observed that male American black bears scent-marked more than females during the breeding season.

To our knowledge, until now, anogenital scent-marking and its association with the estrous status in female ursids is well-described only in the giant panda (Swaisgood et al. 2000, Lindburg et al. 2001, McGeehan et al. 2002, Nie et al. 2012). Chemical analysis of the scent marks from the giant pandas has shown that they include a number of compounds that can be used to identify the gender and the individual identity of the depositing animal (Hagey and Macdonald 2003). Of note, the giant panda is the only bear to have a well-developed anogenital scent gland (Zhang et al. 2008), although anal scent sacs have been documented in brown bears (Rosell et al. 2011). However, no information is available on the presence of anal glands or scent sacs in sloth bears. Studies on estrous behavior in captive populations of giant pandas have shown that female giant pandas increase scent-marking through anogenital rubbing and/or urination during estrus (Swaisgood et al. 2000), with rates of marking peaking about 4–8 days prior to ovulation (Lindburg et al. 2001). Nie et al. (2012) found evidence suggesting that free-ranging female giant pandas also scent-marked more during the breeding season. Our data from the female sloth bears indicate that the females scent-mark almost exclusively during breeding season through genital rubbing.

The influence of the proximity to males on the estrous cycle of females has been demonstrated in many species of domestic animals (Rekwot et al. 2001), as well as wild animals, such as the bandicoot rat (*Bandicota bengalensis;* Sahu and Ghosh 1982) and cotton-top tamarins (*Saguinus oedipus;* Widowski et al. 1992). The presence of a male in the same captive enclosure with a female has been shown to increase the probability of cycling in female sun bears (*Helarctos malayanus;* Frederick et al. 2013). There are, however, no data available on the effect of proximity to males and other females on reproductive behavior and scent-marking by female sloth bears. Our results indicate that physical access to a greater number of males during estrus can increase the occurrence of genital rubbing in female sloth bears. However, the presence of other females, both with and without direct physical access, does not affect this behavior. This finding provides evidence to support our hypothesis that genital rubbing in the sloth bear is a form of scent-marking used for intersexual communication. Although the *P*-values for the influence of proximity to males in some of our analyses were slightly above our threshold of 0.05, the overall results indicate the positive influence of male proximity on the genital rubbing by female bears in estrus (Amrhein et al. 2019).

Female brown bears are known to reproductively senesce with advancing age, especially after 15 years of age (Schwartz et al. 2003) and giant pandas show a dampened expression of estrus-associated marking behavior with age (M.A. Owen, unpublished data). The significant negative correlation of age with genital rubbing in female sloth bears might be a subtle indicator of reproductive senescence, similar to brown bears and giant pandas. Another possible explanation is the reduction in the overall activity levels of the sloth bear females with advancing age, as observed in giant pandas (Kleiman 1983).

The odors from the scent-markings of females in estrus are known to strongly influence and arouse sexual behavior in males of mammalian species (Ferkin et al. 2004, Cerda-Molina et al. 2006) and this pattern is well-established in giant pandas (Swaisgood et al. 2000, 2004). Our finding of the high rate of occurrence of genital rubbing by female sloth bears during estrus indicates that this might be one of the key factors in chemical communication via scent-marking for attracting a male partner in wild populations during the breeding season. Studies on polar bears (*Ursus maritimus*) have shown that the sex and reproductive status of conspecifics is conveyed via pedal scent, suggesting that scent is important for finding mates (Owen et al. 2014). Mating success can be a critical factor in population viability and ultimately survival of a

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species (Molnár et al. 2008), and sloth bear populations in the wild have patchy distributions (Yoganand et al. 2006); therefore, the role of this female scent-marking behavior may be vital in their reproduction dynamics. The evident effects of age, and the proximity to males, are thus of great importance. In addition, further studies are needed on the reproductive behavior and physiology of sloth bears, as well as density-dependent effects on their reproductive success in the wild.

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