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Ontogeny of scent marking behaviours in an apex carnivore

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Abstract

Puma (*Puma concolor*) communication with conspecifics is via indirect scent marking behaviours that are important for individuals to advertise their territory and reproductive status, but little is known about how the behaviours develop with age. To examine the development of scent marking behaviours, we monitored the behaviours of adult pumas and dependent kittens. Based on video recordings, we found that the frequency of puma communication behaviours significantly changed over time. Kittens exhibited olfactory investigation more frequently as they aged, but kittens generally did not exhibit scent marking behaviours. Kittens travel with their mothers until they disperse, so there is no need to establish territories or advertise availability to mate, but kittens are at risk of injury or mortality from other pumas. It is possible that there is no functional need for dependent kittens to scent mark until they mature, but there is a need for frequent use of investigative behaviours.

Keywords

behaviour, development, *Puma concolor*, ontogeny, reproduction, scent marking.

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1. Introduction

Ontogeny (the development of an animal) is shaped by physical changes and conditions throughout the animal's life; while behavioural ontogeny is the course of development that a young animal undergoes to refine its behaviours in order to survive as an adult (Immelmann, 1980). The origins of behaviours are often complex due to a multitude of external and internal factors (e.g., genetic heritability, environmental conditions) facilitating their development (Bateson, 1981) and whether the behaviours are innate (behaviours that do not require learning) or learned (behaviours that require learning and experience). While individual variation in behaviours may occur due to differential experiences (e.g., stronger fear response to certain stimuli), most behaviours are dictated by life history and must develop properly to allow the animal to acquire resources and create progeny.

Among solitary carnivores, much of the communication and interactions with conspecifics is reliant on indirect scent marking behaviours (the action of depositing volatile chemical compounds via defecation, urination, or glandular secretions; Gorman & Trowbridge, 1989). Many solitary carnivores will scent mark to advertise territory and reproductive status (Kleiman & Eisenberg, 1973; Allen et al., 2016a; Krofel et al., 2017), and scent marking is also believed to be an honest indicator of an individual's health (Zala et al., 2004) and social status (Allen et al., 2015). For many solitary carnivores, affiliative behaviours most often occur between mothers and kittens during the rearing phase (which can last for >1 year in larger species) and briefly between males and females during mating. The long dependency of kittens on their mothers provides ample opportunities for learning important behaviours such as stalking and killing of prey (Bekoff et al., 1984). Despite the importance of scent marking behaviours for communication in many solitary carnivores (Allen et al., 2016a), little is known about their ontogeny.

Pumas (*Puma concolor*) are a large, solitary felid whose intraspecific communications are primarily conducted via indirect scent marking behaviours. Scraping is the most frequent type of scent marking behaviour for pumas (Harmsen et al., 2010; Allen et al., 2014), with less frequent behaviours including urine marking, body rubbing, and rolling (see Table 1 for descriptions; Allen et al., 2014), as well as investigative behaviours of olfaction and the flehmen response. Puma scent marking behaviours primarily occur at community scent marking areas (i.e., 'community scrapes'; Allen et al., 2014). Adult male pumas frequently visit community scrapes to

Table 1.
An ethogram describing the communication (scent marking and investigative) and behaviours known to be used by pumas. We also provide a prediction for if the behaviour will be exhibited more or not as kittens develop.

Behaviour	Description	Prediction
Urine marking	The puma sprays urine on the ground or a scrape previously made by another puma.	Because urine marking involves a basic bodily function, the behaviour could be exhibited by young kittens. However, the purpose urine marking is to advertise status for potential rivals and mates and will be displayed more often as pumas age, and less often in kittens than in adults.
Scraping	The puma scrapes with their hind feet to create a depression and a scraped mound of material and sometimes urinates and/or defecates on it.	Because scraping is used by pumas to advertise status for rivals and potential mates, the behaviour will be displayed more often as pumas age, and less often in kittens than in adults.
Olfaction	The puma sniffs a scrape previously made by another puma.	Because the sense of smell is used as soon as a kitten is born, the behaviour will be innate and will not be displayed more often as pumas age or in different proportions from adults.
Flehmen response	The puma lifts its head and curls back its neck to investigate scent with its vomeronasal organ.	Because the flehmen response is used by pumas for in-depth investigation of scent left by potential mates or unknown pumas, the behaviour will be displayed more often as pumas age and less often in kittens than in adults.
Rolling	The puma rolls back and forth on the scent marking area, sometimes called "vegetation flattening" for other species.	Because rolling is used primarily by adult males (Allen et al., 2016b), the behaviour will be displayed more often as pumas age and less often in kittens than in adults.
Body rubbing	The puma rubs its cheek, neck, or shoulder on an object (ground, stump, tree branch, etc.) at the scent marking site.	Because tactile sense is used between kittens and mothers to nurse as soon as the kittens are born, the behaviour will be not displayed more often as pumas age or in different proportions from adults.

advertise their use of the area to potential mates and competitors (Allen et al., 2016b), and this allows females to assess potential mates without risking dangerous encounters with males for themselves and their dependent kittens (Allen et al., 2015). Female visits are thus generally infrequent except for short periods of high activity prior and during oestrus when they are selecting mates (Allen et al., 2015). While dependent kittens do visit community scrapes, it is unclear when scent marking behaviours begin and whether they change over time as kittens age and develop.

Our objective was to examine scent marking behaviours exhibited by dependent kittens of different age classes to determine if scent marking behaviours develop as kittens age and if kittens display the behaviours in different proportions from adults. We hypothesized that pumas would develop marking behaviours as they age, but would use investigative behaviours innately from young ages. We used video camera traps to document scent marking behaviours of dependent kittens at community scrapes and then tested specific predictions for each scent marking behaviour (Table 1).

2. Materials and methods

2.1. Study area

Our study area encompassed approximately 1700 km^2 in the Santa Cruz Mountains of California. The Santa Cruz Mountains run from the San Francisco Bay south to Monterey Bay, with the Pacific Ocean to the west, and Highway 101 to the east. The climate is Mediterranean, with hot, dry summers, and wet, cold winters. Vegetation characteristics in the Santa Cruz Mountains are dominated by redwood forests, and specifics have been described in detail elsewhere (Wilmers et al., 2013). Our study area is highly fragmented by exurban development (Wang et al., 2015), with the cities of San Jose to the north and Santa Cruz to the south, and an arterial highway (California Highway 17) bisecting the study area.

2.2. Field methods

As part of our larger study on puma ecology and conservation, we captured and placed collars (Model GPS Plus 1D, Vectronics Aerospace, Berlin, Germany) on 66 pumas over the course of our study. Our protocols for the capture of pumas followed the guidelines outlined by the American Society of Mammalogists (Sikes et al., 2011) and were approved by the Institutional

Animal Care and Use Committee of the University of California, Santa Cruz (Protocols Wilmc0709 and Wilmc1101), and the Wildlife Investigations Lab of the California Department of Fish and Wildlife. Our protocols for puma capture and handling protocols have been described in detail in Wilmers et al. (2013), and no pumas were killed or sacrificed as part of our research methods.

We monitored the scent marking behaviours of pumas between May 2011 and May 2016 using motion-triggered video camera traps (Bushnell Trophy-Cam, Overland Park, KS, USA) placed at 26 community scrapes (Figure 1). A scrape is the scent mark created by an individual puma, whereas a community scrape is a concentrated location with multiple scrapes ($\geqslant$3 in 9 m^2) used by multiple pumas (Allen et al., 2016b). We identified potential community scrapes during fieldwork and also systematically using an algorithm that alerted us to areas visited by collared pumas multiple times with $>$1 week between visits (Allen et al., 2014). We programmed cameras to record 60 s videos with a 1 s refractory period to maximize recording of behaviours and performed maintenance on the camera traps every 1–3 months depending on battery type and memory card capacity. We monitored each community scrape on average 2.72 $\pm$ 0.23 SE years.

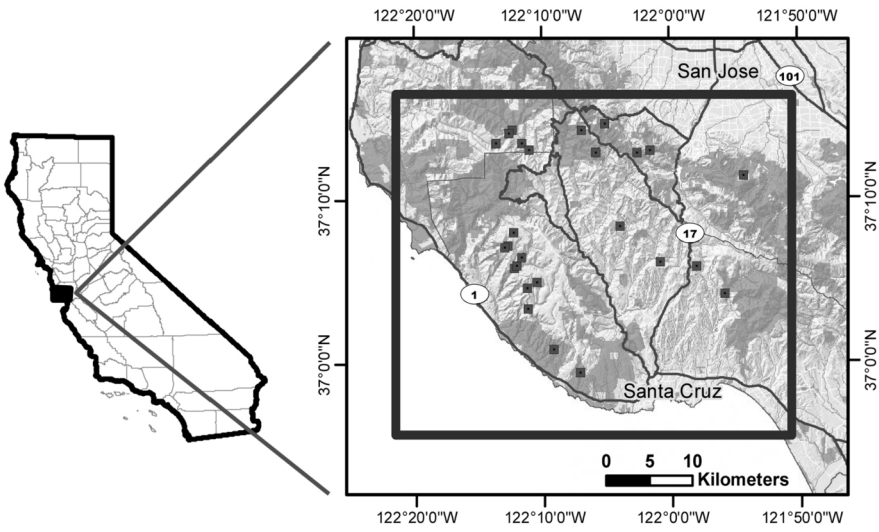

Figure 1. The study area in the Santa Cruz Mountains, showing the community scrapes we monitored for this study.

2.3. Statistical analyses

For the purposes of this study, we focused on visits of dependent kittens to evaluate how scent marking behaviours vary and develop with age. We identified dependent kittens by having spotted coats (which is distinctive to dependent pumas; Logan & Sweanor, 2001), or during visits when they were ravelling with their mother (which generally indicated an age of less than 18 months; Logan & Sweanor, 2001). We used known birth dates for marked pumas. For unmarked pumas, we had 3 trained researchers look at each video to reach a consensus on the age class (0–6 months, 7–12 months, 13–18 months) based on external characteristics (e.g., https://www.cougarnet.org/files/5714/2092/0558/PumaFieldGuide.pdf). For each visit, we recorded the date and time, length of visit to the closest second, and whether scent marking behaviours were displayed. The lead author scored each video for the scent marking behaviours based on descriptions in the ethogram in Table 1.

For our comparisons with adults, we removed adults where we could not confidently determine sex. We also removed consorting pairs ravelling together and females that were travelling with kittens, as these behaviours influence the type and frequency of marking behaviours in adult pumas (Allen et al., 2015). To compare dependent kittens to adult males and adult females, we used generalized linear models, with whether the given behaviour was exhibited or not as the dependent variable and the puma age class as the independent variable. To compare the exhibition of behaviours among kitten age classes, we used a chi-square analysis for olfaction and Fisher's exact tests for all other behaviours due to small sample sizes.

3. Results

We monitored 26 community scrapes for a mean ± SE of 991 ± 83 days (range 306–1651). We documented 678 visits by adult male pumas and 223 visits by adult female pumas ravelling without kittens. We documented visits by 78 kittens; 16 visits by kittens 0–6 months old, 38 visits by kittens 7–12 months old, and 24 visits by kittens 13–18 months old.

Dependent kittens exhibited olfactory investigation at 57.7% of visits (Figure 2), significantly less than adult females (75.3%, $\beta = 0.81 \pm 0.28$, $p = 0.004$) or adult males (76.1%, $\beta = 0.85 \pm 0.25$, $p = 0.0005$). Kittens exhibited olfactory investigation significantly more frequently at older age classes ($\chi^2 = 5.83$, $p = 0.05$), using olfactory investigation at 31% of visits

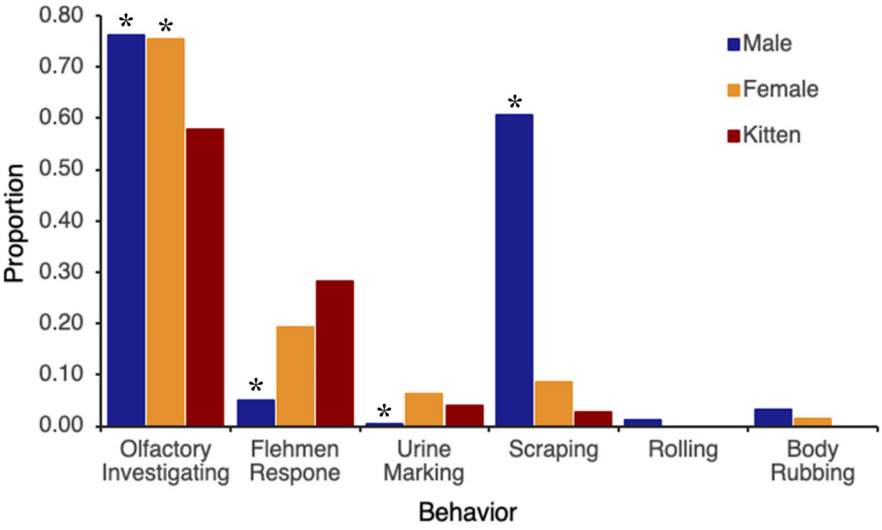

Figure 2. Proportions of behaviour exhibited by dependent kittens, adult males, and adult females. Asterisks note the behaviours that were significantly different than dependent kittens.

at 0–6 months of age, but 66 and 63% of visits at 7–12 and 13–18 months of age, respectively (Figure 3).

Dependent kittens exhibited the flehmen response at 28.2% of visits (Figure 2), significantly more than adult males (5.0%, $\beta = -2.01 \pm 0.31$, $p < 0.0001$), but not significantly different than adult females (19.3%, $\beta = -0.50 \pm 0.30$, $p = 0.10$). Kittens exhibited flehmen significantly more frequently at older age classes ($\chi^2 = 8.67$, $p = 0.01$), using olfactory investigation at 19% of visits at 0–6 months of age, but 32 and 29% of visits at 7–12 and 13–18 months of age, respectively (Figure 3).

Dependent kittens exhibited urine marking at 3.8% of visits (Figure 2), significantly more than adult males (0.4%, $\beta = -2.20 \pm 0.83$, $p = 0.008$) who rarely exhibited the behaviour. Kittens were not significantly different than adult females (6.3%, $\beta = 0.52 \pm 0.65$, $p = 0.42$), and kittens did not vary in their use of urine marking among age classes ($p = 0.59$, Figure 3).

Dependent kittens exhibited scraping at 2.6% of visits (Figure 2), significantly less than adult males (60.6%, $\beta = 4.07 \pm 0.72$, $p < 0.0001$), but not significantly different than adult females (8.5%, $\beta = 1.26 \pm 0.76$, $p = 0.09$). Kittens did not significantly vary in their use of scraping behaviour ($p =$

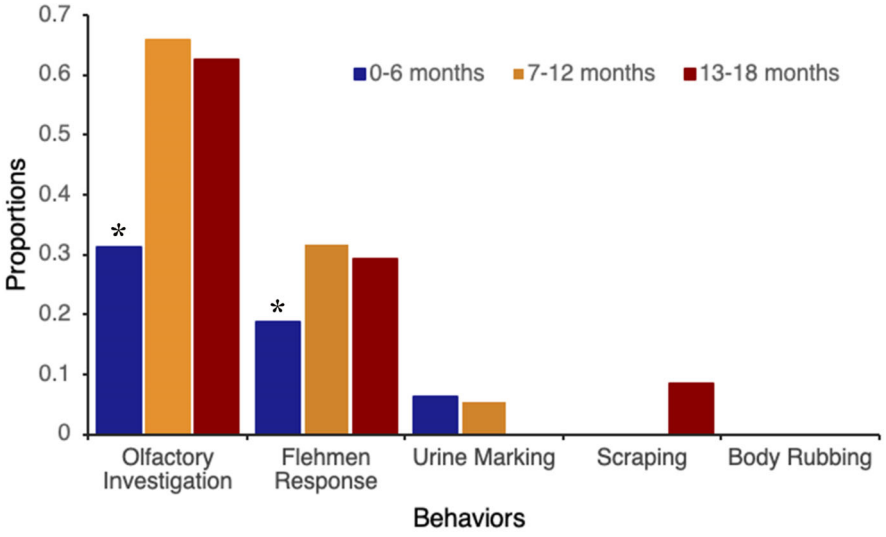

Figure 3. Proportions of behaviour exhibited by dependent kittens of different ages. Asterisks note age classes that were significantly different than others.

0.13) but never exhibited scraping from 0–12 months of age while using scraping at 8% of visits from 13–18 months of age (Figure 3).

Dependent kittens did not ever exhibit body rubbing or rolling (Figure 2), which precluded them from statistical comparisons. Adult females also never exhibited rolling behaviour but exhibited body rubbing behaviour at 1.3% of visits. In contrast, adult males exhibited rolling at 1.2% of visits and body rubbing at 3.1% of visits.

4. Discussion

Our results show that many communication behaviours by puma kittens at community scrapes vary dependent on age, highlighting the importance of understanding the ontogeny of these behaviours. There was a general pattern of investigative behaviours being exhibited more frequently than marking behaviours by dependent kittens. This may be related to the ontogeny of the behavioural development, or it may be a function of kittens learning to interact with their environment to influence their fitness later in life. Most of the behaviours were exhibited in different proportions than adults, but dependent kittens also exhibited most of the behaviours at young ages, showing they are capable of the behaviours even if they are not used frequently. The two

exceptions were body rubbing and rolling behaviours that dependent kittens never exhibited at community scrapes during this study. Rolling is primarily a male behaviour while body rubbing is exhibited by both sexes. However, both behaviours are infrequently used and the reason behind the behaviours is generally unknown (Allen et al., 2016b). The variation and development of these behaviours, and their comparisons with adult pumas of both sexes, highlights the role they play in the behavioural ecology of the species. At the same time, dependent kittens are also developing other aspects of social behaviour, including exchanging tactile, auditory, and other cues, although we noted only very limited instances. Our study was limited by small sample sizes and future studies could further examine the ontogeny of puma communication and social behaviours.

Felid scent marking behaviours vary by sex (Miyazaki et al., 2006; Allen et al., 2014), and this could affect the behaviours we observed in kittens. The dependent kittens in our study generally exhibited behaviours in proportions more similar to adult females than males, which may indicate learned behaviour but is more likely a reflection of behavioural ecology rather than functional ability. Male and female pumas are born in the same proportions (Logan & Sweanor, 2001), but the sexes of the dependent kittens in our study were unknown and male and female kittens may act differently. But given the similarity to female behaviours, it is possible that the strategies of the kittens are similar regardless of sex. Kittens exhibiting investigative behaviours would allow them to assess the area to determine if any adult males are in the area without scent marking to make their presence known, similar to subadult pumas or adult females (Allen et al., 2015) that prefer to remain unknown to territorial males due to threats of aggression and infanticide (Logan & Sweanor, 2001). Kittens will travel with their mothers until they disperse at around 18 months (Logan & Sweanor, 2001), and there is no need to establish territory, dominance, or advertise availability to mate during this time. Females occasionally scent mark while traveling with kittens, and kittens therefore are more exposed to the behaviours more frequently used by females than males. Dependent kittens also may not scent mark or scrape as there is no need to diminish kin recognition for inbreeding avoidance while still in their natal home range (Coombes et al., 2018). It may be that the behavioural dynamics outweigh functional needs of dependent kittens to scent mark, despite their ability to scent mark if needed.

Dependent puma kittens exhibited olfactory investigation less frequently than adults but exhibited the flehmen responses more frequently, and substantially increased their use of both behaviours at later age classes. Our findings were contrary to our hypothesis that the age of a kitten would not influence its frequency of olfactory investigation, as kittens are capable of olfactory investigation early in life. However, kittens did use olfactory investigation at approximately one third of their visits at 0–6 months of age with frequency increasing as the kittens aged, suggesting that the behaviour may be innate (behaviours that do not require learning) but continues to develop over time. We also found that kittens used the flehmen response more frequently than adults and exhibited the flehmen response more often at older age classes. Felids use the flehmen response for more complex interpretation of scent with their vomeronasal organ (Hart & Leedy, 1987). The increasing use of both investigative behaviours as the kittens age may be the result of increasing curiosity or to develop their olfactory memory. Both aspects of their behavioural ecology (curiosity and memory development) are important as kittens age while traveling large distances in their mother's home range.

Our documentation of scent marking behaviour showed that dependent kittens are capable of scent marking and do occasionally scent mark at community scrapes, but generally did not exhibit scent marking behaviours. While instances of urine marking may be dismissed as simple evacuation of the body, the urine markings we documented occurred on scrapes previously made by other pumas, similar to how female pumas urine mark at scrapes made by territorial males when in oestrus (Allen et al., 2015). Scraping was not significantly different among puma age classes, except that dependent kittens less than a year old did not ever exhibit scraping behaviour. Females that visit community scrapes with kittens do not frequently scrape (Allen et al., 2015), suggesting that kittens lack a model to base the behaviour on. Furthermore, while young dependent kittens may visit community scrapes, the frequency in their visits is lower than older kittens. It is possible that females bring their kittens to scrape sites at different ages for development of behaviours or that traveling with their mother becomes more frequent over time for kittens in concordance with risks. In addition, scent marking by kittens occurred occasionally, yet scraping was not done until kittens were at least 13 months old, suggesting that scent marking behaviours do not develop until later in life. It is possible that females who scrape more frequently act

as a model for their kittens to scrape more frequently, but this was not something we could evaluate in this study due to low sample sizes. Kittens may become more sensitive to signals from scent marks as they must learn how to assess the quality of potential mates and establish their own territory; however, it is also possible that we under-detected scent marking due to small sample sizes, and future studies should re-evaluate this.

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