ORIGINAL ARTICLE

Investigating scent communication and latrine use to inform translocation of the Tasmanian devil

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Abstract

Translocation programs for endangered mammalian carnivores face numerous challenges, including the potential for lethal inter-actions between releasees and resident conspecifics. Here we evaluate whether familiarizing residents with the scent of releasees might be an effective strategy for reducing aggression following Tasmanian devil (*Sarcophilus harrisii*) translocation. Tasmanian devils live in loose social networks in which residents have overlapping home ranges and use communal latrines. In a controlled ex situ experiment, we first found that devils readily distinguished between the feces of familiar and unfamiliar conspecifics. Next, we swapped feces between devils slated for release and wild resident devils, seeding a random half of communal latrines in the wild with the feces of releasee prior to their release, and used motion-activated video cameras to record devil visits. Wild resident devil visitation frequency and olfactory communication behaviors initially increased at experimental latrine sites where feces were deposited but not at control sites. After release, the translocated devils used both types of latrine sites and spent more time investigating experimental sites, but investigatory and scent-marking behaviors of the wild resident devils decreased, suggesting that they had become familiar with the releasees through scent. We further show that Tasmanian devil latrine sites are strongly associated with ecotones and the presence of shrubs. These findings contribute to our understanding of Tasmanian devil scent ecology and suggest that devil translocations conducted into existing populations would benefit from familiarization through latrine scent manipulation. More broadly, our results suggest an important role for scent ecology in carnivore translocation programs.

Significance statement

A conservation management action that is being trialed for endangered Tasmanian devils is to breed devils in human care and reinforce dwindling small resident populations through translocation. However, with limited social interactions, unfamiliar devils may interact aggressively, increasing injury and reducing translocation success. Scent communication is nearly ubiquitous in mammals and may be a prerequisite for management of social aggression. Almost nothing is known about devil scent ecology. Our discovery that devils become familiarized with conspecifics through feces, combined with findings that wild devils that are exposed to translocated devil scent in advance of release show reduced interest in scent-seeded latrines, even after new devils are released, suggests that devils familiarized through scent in advance of relocation may exhibit reduced aggression. These results provide an avenue for improving the success of translocations for devils and other at-risk solitary mammalian carnivores.

Introduction

Chemical signals in mammals play a key role in structuring social organization, influencing ranging patterns, and governing mating decisions and social aggression—among other functions—and thus have great but largely unrealized potential for application in conservation (Swaisgood 2010; Campbell-Palmer and Rosell 2011). One widespread phenomenon often mediated by chemical signals is reduction of aggression towards familiar individuals. Often referred to as the "dear enemy" phenomenon, representative species from most taxonomic groups display less aggressive behavior towards neighbors or familiar individuals (Temeles 1994; Reichert and Quinn 2017). A resident animal may face greater risk

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from an unfamiliar intruder than a known neighbor because a history of agonistic interactions with a known individual has established a stable dominance or territorial relationship. Thus, escalated conflict is avoided with familiar individuals.

In species reliant on chemical communication, scent marks, feces, or other chemical signals are used to distinguish familiar from unfamiliar individuals (Aragón et al. 2001; Ibáñez et al. 2013). Furthermore, a number of studies in mammals (Nakamura et al. 2007; Zenuto 2010) and reptiles (López and Martín 2002) demonstrate that repeated exposure that increases familiarity with an individual's odor alone can reduce aggression when that individual is subsequently encountered.

Serving functions as important as these, animals are predicted to deposit scents in locations strategically to maximize encounter rates with conspecifics and thus ensure that the signal is received (Alberts 1992). Communal scent-marking sites provide one mechanism to maximize the communicative function of chemical signals. At these sites, animals can reliably deposit scents in areas frequented by conspecifics that investigate these signals and often deposit their own. Furthermore, animals often select prominent environmental features to create communal scent stations, which increases the likelihood that the chemical signals are encountered by conspecifics (Zollner et al. 1996; Attum et al. 2006; Barja and List 2006; Balestrieri et al. 2009; Torgerson 2014). Almost without exception, individuals use these sites to communicate with conspecifics that are directly encountered infrequently, thus chemical signals can be used to communicate in absentia.

When chemical signals in communal scent stations primarily comsisted of feces, they are commonly referred to as middens, dungheaps, or latrines. Feces, urine, anal, and other marks from multiple individuals accumulate in latrines and often occur along territorial boundaries, trails, and trail junctions, and in microhabitats that support signal persistence or detection, such as conspicuous objects in relatively open habitats (Alberts 1992; Buesching and Jordan 2019). These chemosignals are typically long-lasting, especially feces and anal secretions, which contain compounds of high molecular weight and low volatility, whereas urine contains more volatiles and tends to be more short-lived (Regnier and Goodwin 1977; Alberts 1992; Apps et al. 2015). Latrine use is a common feature of olfactory communication in a variety of species, including ungulates (Brashares and Arcese 1999; Attum et al. 2006; Wronski et al. 2006; Wronski and Plath 2010; Linklater et al. 2013; Marneweck et al. 2018), lagomorphs (Zollner et al. 1996), placental carnivores (Begg et al. 2003; Palphramand and White 2007; Kilshaw et al. 2009; Mustelidae: Barocas et al. 2016; Herpestidae: Jordan et al. 2007; Canidae: Macdonald 1980; Darden et al. 2008; Barja 2009; Hyaenidae: Gorman and Mills 1984; Procynidae: Barja and List 2006), primates (González-Zamora et al.

2012; Dröscher and Kappeler 2014; Eppley et al. 2016), and marsupial carnivores (Pemberton 1990; Ruibal et al. 2011). Latrine use has been prominent in some lineages dating back to some of the earliest mammalian ancestors (Fiorelli et al. 2013). The widespread and convergent taxonomic use suggests functional importance for communication. The use of latrines serves multiple communication functions, but one common function appears to be mediating social familiarity (Palphramand and White 2007; Dröscher and Kappeler 2014) and territory maintenance (Buesching and Jordan 2019; Claase et al. 2022).

Here, we investigated the role of latrines and scent in social and communication behavior in the Tasmanian devil (*Sarcophilus harrisii*)—the largest extant marsupial carnivore and endemic to the island of Tasmania. Devils live solitarily but encounter conspecifics in overlapping home ranges especially at feeding sites (large carcasses), where significant aggression can occur (Pemberton and Renouf 1993). Similar to other carnivores, devils place scent (via urine, feces, anal drags, and the scent gland itself; Pemberton and Renouf 1993) at communal latrines suggesting a possible role for social communication (Pemberton 1990; Shier et al 2019a, b) Together, these observations indicate that devils live in a social network comprising familiar individuals that encounter each other periodically, yet the extent and function of these social relationships remain largely unknown. However, there is some evidence that aggression is highest when individuals first encounter one another, and it gradually diminishes through time, that they establish "truce relationships" among individuals that have regular contact, and that unfamiliar intruders are attacked by residents (Buchmann and Guiler 1977).

Once numerous, Tasmanian devils have declined by approximately 77% in affected areas (Lazenby et al. 2018) due primarily to a contagious cancerous tumor—devil facial tumor disease (DFTD)—placing the species at risk of extinction (IUCN listed as endangered; Hawkins et al. 2008). In the absence of methods to control DFTD, management actions have focused on ways to reduce anthropogenic sources of mortality (Lawrence and Wiersma 2019) and mitigate the negative effects of a small population size (Grueber et al. 2018; Lazenby et al. 2018). Thus, more recently, reinforcement translocations (release of ex situ bred animals into existing populations of wild resident devils) have been trialed for genetic and demographic rescue in areas with low-density devil populations (Fox and Seddon 2019). However, translocating devils into established populations may pose new challenges.

By nature, translocations entail social disruption in which unfamiliar individuals meet in novel circumstances. Lack of social familiarity and risk of aggression can negatively impact release outcomes (Shier 2006; Linklater and Swaisgood 2008; Shier and Swaisgood 2012), and a period of social integration may be required before translocated

individuals can settle and attain normal foraging patterns and reproductive success (Gusset et al. 2006; Poirier and Festa-Bianchet 2018). Even in areas with no resident population, members of the release group(s) may have varying familiarity with other members. In reinforcement translocations, released individuals must contend with repeated encounters with unfamiliar individuals already occupying and often defending the area (Shier et al. 2019a, b). Residents often respond with heightened aggression to translocated intruders. This social disruption can cause stress to both groups, risks injurious aggression, and can be an obstacle to successful settlement, survival, social integration, and future reproduction. Thus, aggression between unfamiliar individuals may be an important obstacle to translocation success.

In devils, social disruption could also cause an increase in bite-mediated DFTD transmission (Hamede et al. 2013) by increasing post-release movement and aggressive encounters between conspecifics. An underutilized tool to address the problem of social unfamiliarity in translocations is the use of communication signals prior to release to familiarize residents with released individuals and vice versa (Shier et al. 2019a, b). We studied several aspects of Tasmanian devil scent ecology to inform future translocations.

1. To determine whether devils discriminate between the odors of familiar versus unfamiliar conspecifics, we conducted a controlled experiment with devils ex situ. The ability to discriminate familiarity on the basis of these odors is a prerequisite for management of social aggression between unfamiliar individuals using odor familiarization tools.
2. By comparing habitat features at latrine sites with control sites, we sought to determine how devils select sites for latrine establishment. We predicted that devils would select conspicuous features in the environment and/or prominent locations where encounter rate of their chemical signals with other devils can be maximized. Furthermore, latrine site features can be used to guide establishment of artificial latrines to manipulate behavior in conservation contexts.
3. To explore the potential for mediating aggression between ex situ managed devils that were selected for release (releasees) and wild resident devils during translocation, we experimentally manipulated resident latrines by depositing feces from releasees at randomly chosen 50% of established latrines before they were released at the site. We predicted that visitation and scent communication by resident devils would initially increase at experimental sites and then decrease over time as residents became habituated to release devil scent. We also presented releasees with the feces of wild resident devils to familiarize them with the scent of residents prior to release.

Methods

Experiment 1: Effect of familiarity in ex situ managed devils

To evaluate whether individual devils could discriminate between familiar and unfamiliar conspecifics, we compared the investigation rates of devils in response to conspecific feces.

Subjects

We tested each of $N = 12$ focal subjects (6 males: 6 females) in two trials, one with feces from male donors and one with feces from female donors. Feces were collected from 24 scent donors, 6 familiar males, 6 familiar females, 6 novel males, and 6 novel females. All female donors were non-reproductive. Familiar conspecifics were housed within one enclosure of the focal devil and unfamiliar conspecifics were housed in a different ex situ facility. To minimize pseudoreplication, donor scents were used only twice, once with a focal female subject and once with focal a male subject.

Scent collection

Donor feces were collected directly from individual enclosures by keepers during routine husbandry procedures. Staff wore latex gloves and stored individual feces in Ziploc bags that were immediately placed into a –18 °C freezer. Individual samples were labeled with the individual, sex, and date of collection. Due to changes in the protocol to account for facility limitations and animal cooperation, some donor samples had to be collected during the duration of the study; and thus, the time an individual sample spent in the freezer was not standardized. However, freezing should arrest most odor-related deterioration, and we included feces age as a covariate in the analyses as a precaution (see "Data analyses" section below). Feces age varied from 1 to 72 days.

Procedure

We simultaneously placed the feces of (1) a familiar conspecific and (2) an unfamiliar conspecific into the focal devil's enclosure 1 m apart and turned on a video recorder to begin the 10-min test. During all trials, an observer stood outside the enclosure and recorded behaviors are listed in Table 1; thus, it was not possible to record data blind. At the end of testing, we reviewed videos to ensure accuracy.

Data analysis

We computed two composite variables: (1) investigation, defined as a combination of time spent sniffing, handling, and mouthing; and (2) marking, defined as a combination of time spent anal dragging (over the donor scent), defecating, urinating, and countermarking. We employed a multi-level (nested) mixed effects regression model to determine whether devil scent communication (duration investigation or marking) was affected by donor type (familiar or unfamiliar), sex of the donor devil, or the interaction of these factors with individual identification included as a random effect. We also examined whether scent communication was influenced by the interaction between the sex of the subject and donor devil. To account for the potential impact of donor feces age, we included it as a covariate in each of the models along with sex and feces type (familiar vs unfamiliar). All data analyses were conducted in STATA14 (StataCorp LLC, College Station, Texas).

Field survey: Wild latrine habitat selection

During June of 2015, we established forty-eight belt transects across the four habitat types in Narawntapu National Park, Tasmania (41.1487° S, 146.6039° E; see Fig. 1 for the location of Narawntapu National Park): (1) coastal scrub $N = 12$; (2) forest $N = 12$; (3) open grassland $N = 12$; and (4) ecotone edge, $N = 12$, to quantify the types of habitat devils select for latrine sites. Origin points for each of the 12 transects within each habitat type were randomly generated using ArcGIS 10.3. We used a random number generator to select the bearing angle for each transect from an azimuth between 1 and 360. Transects extended 200 m from each origin point, yielding a minimum distance between transect origins of 250 m. We visually scanned 2 m on either side of the centerline, for a total belt width of 4 m.

Table 1 Ethogram of devil behavior

Behavior	Definition
Anal drag	Devil drags anal gland across ground or other feces
Countermark	Urinates or defecates on top of another feces already present
Defecate	Passing of fecal material
Handle	Handles another feces that is already present at the latrine
Mouth	Handles another feces that is already present at the latrine
Mouth gape	Mouth partially (half gape) or fully (full gape) open, showing teeth in a defensive posture
Sniff	Head lowered, nose near to the ground (about 5 cm or less), accompanied by sniffing and/or tasting
Urinate	Eliminates urine

We used a number generator to randomly select three control points along each transect (1–200 m). At each control point observers recorded the following data: GPS location, elevation (m), percent slope and aspect, dominant community type (coastal scrub, forest, grassland, and/or ecotone, where ecotone was defined as the transition zone between the forest and open grasslands), dominant species of grass, forb, shrub (if codominance, we recorded both species), and distance to human-built structure. We recorded 1 km for all transects with human built structures farther than 1 km from the transect. Visual estimates were recorded for vegetation cover and height over a 5 × 5 m plot centered on the control point. Within plots, we estimated the following: (1) percent bare ground, (2) percent of vegetation < 0.1 m high, (3) percent of vegetation 0.1–1 m high, (4) percent of vegetation 1–5 m high, and (5) percent of vegetation > 5 m high. All latrines had a slope of less than 1%; and therefore, we did not attempt to estimate slope.

If Tasmanian devil scat was detected along a transect, we searched the immediate area in 3-m concentric rings for additional feces. Observers continued along the transect unless three or more feces were found within 3 m of each other, in which case, the area was designated as a devil latrine site. Based on observations of devil latrines in the wild, latrines were considered independent if there was ≥ 20 m between feces. GPS points were taken at the center of the latrine and for each individual feces. The number of fresh (< 1-week-old) and old (> 1-week-old) feces was recorded as well as the type of substrate on which the feces was found (bare ground, gravel road, grass, or forb). We also recorded whether the latrine was located on a single trail/road or if it occurred at an intersection. We established a 5 × 5 m plot around the center of the latrine once all the feces were recorded. For each of the latrine plots, we recorded the same parameters as listed above for control plots.

Data analysis

We used Fisher's exact test to examine the relationship between habitat type and the presence of a latrine. Because all but one latrine was found in ecotone habitat, we restricted the analysis for the remaining variables to ecotone plots ($n = 52$). Multivariate logistic regression models were used to determine which habitat variables were associated with latrine plots within ecotone habitat based on Akaike's information criteria (AIC). We used backwards stepwise removal of predictors with a criterion to remove ΔAIC less than 2 AIC units. Possible predictors were as follows: elevation (m), presence or absence of grass, presence or absence of forbs, presence or absence of shrubs, distance to human structure (m), percent cover of bare ground, percent cover of vegetation < 0.1 m high, percent cover of vegetation 0.1–1.0 m high, percent cover

Fig. 1 Aerial imagery showing (**A**) the location of the study area in Narawntapu National Park, Tasmania (white placemark), and expanded views of the study area showing (**B**) the locations of experimental (red placemarks with "E" labels) and control latrines (blue placemarks without labels) used in experiment 2, and (**C**) the locations of latrines found in the transect survey (white placemarks). Geomapping and aerial imagery from Google Earth Pro (Google LLC)

of vegetation 1–5 m high, and percent cover of vegetation > 5.0 m high. Transect was modeled as a random effect. All data analyses were conducted in STATA14.

Experiment 2: Effects of pre-release scent familiarization on the behavior of wild resident and translocated devils

This was the first translocation into mainland Tasmania; and thus, the resident devils were wild-born.

Experimental design

We selected 12 devil latrines within Narawntapu National Park for this study (Fig. 1b). All selected latrines were active (> 2 fresh feces) and distributed evenly across the park. We randomly assigned each latrine to an experimental (scented, $n = 6$) or control (unscented, $n = 6$) treatment. All 12 latrines were monitored from June to early November 2015 for a total of 20 weeks divided into three time periods

(pre-manipulation: 6 weeks; manipulation: 8 weeks; postmanipulation: 6 weeks; Fig. 2). During the 6 weeks of premanipulation monitoring, we collected $n = 72$ feces from many locations throughout the park, making sure to avoid collection within the 12 designated latrines. In addition, we collected $n = 216$ from the two large (22 ha) free-range enclosures (FREs) where the releasees were being housed ex situ. Immediately after collection, we stored all feces in a –20 °C freezer until use. Following the 6-week pre-manipulation monitoring period, we manipulated the experimental latrines by adding feces collected from ex situ managed devils selected for release from FREs. During this 8-week period, we placed scent (4 feces/week; 2 from each of the 2 FREs) into each of the six experimental latrines. Simultaneously, we placed scent (6 feces/week) collected from wild latrines in Narawntapu into each of the two FREs. Scent was presented in a circular pattern with each feces located approximately 1 m from each other to simulate a wild devil latrine. In addition to familiarizing the wild resident devils with the scent of the releasee devils and the releasees with wild resident devil scent, we familiarized the releasees
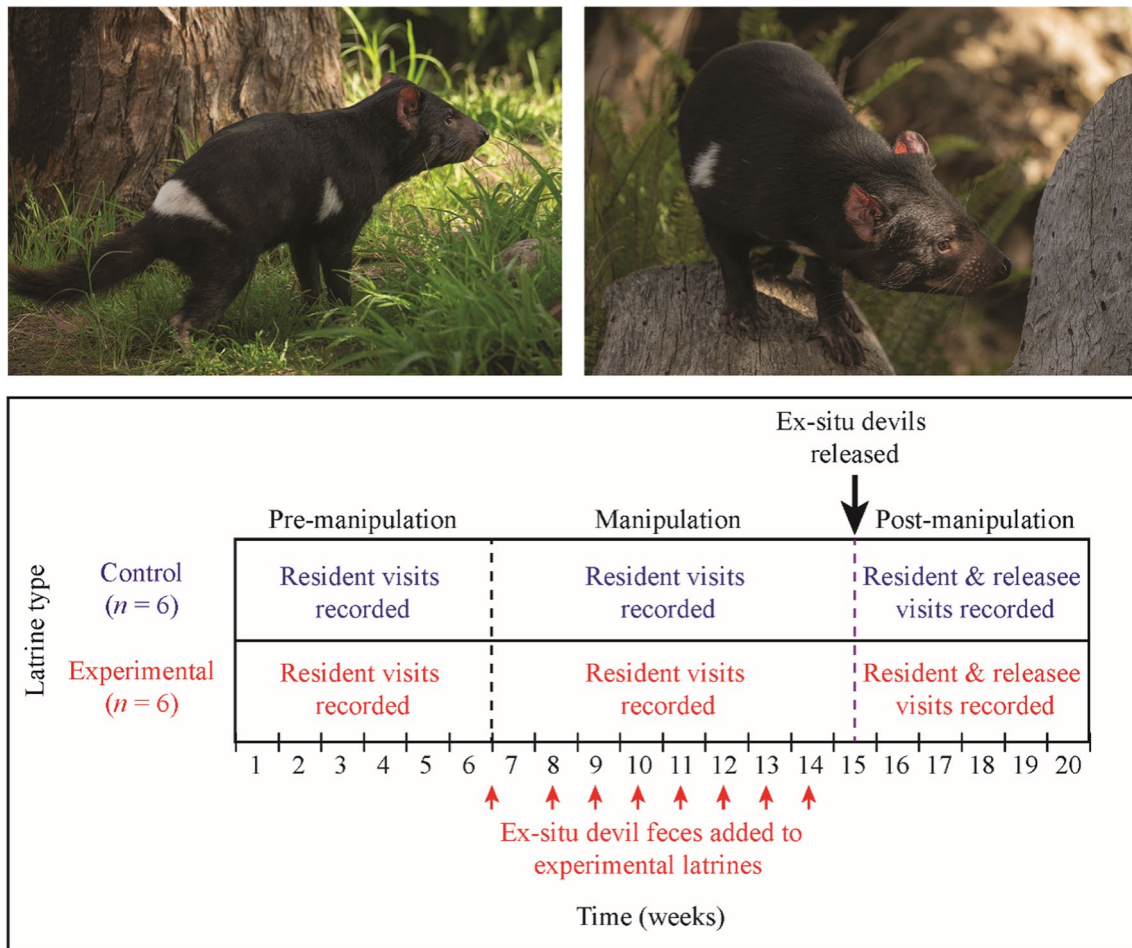
Fig. 2 Experimental design for experiment 2. Photo credit: San Diego Zoo Wildlife Alliance

housed in the 2 FREs with each other's scent. To do so, every week during the manipulation period, we swapped six feces between FREs.

We monitored all latrine sites using remote cameras (Faunatech: Reconyx hyperfire) set to take 15-s videos with a 1-s trigger lapse period. This allowed us to document entire visits as devils continued to trigger cameras as long as they remained in the field of view. We downloaded data from memory cards weekly.

On 25 September 2015, 20 ex situ managed Tasmanian devils (11 males and 9 females) were released into the park, beginning the post-manipulation (post-release) period. Devils in the release cohort were of varying ages and had been managed in human care for at least two years. While we ceased all scent manipulations post-release, we continued to monitor all 12 latrines for another 6 weeks. Residents as well as releasees were identified based on unique pelage coloration patterns where possible. We reviewed all videos and images generated and quantified the same behaviors as in experiment 2 (Table 1). In addition, we quantified the total visit duration for each devil visit to a latrine.

Data analysis

To test for the effects of scent manipulation on the frequency of devil visitation and marking, we constructed separate GLMMs with total frequency of visits or marks during the manipulation period as response variables and treatment and pre-manipulation visitation or marks as fixed effects. We were not able to compare the frequency with which residents visited the latrines in the pre-manipulation and manipulation periods to the post-manipulation period because we were not able to distinguish residents from releasees in $n = 41$ of 182 post-manipulation camera trap videos (i.e., the frequency of resident visits in the post-manipulation period is unknown). This was not an issue for duration data as we simply dropped the 41 latrine visits by unknown devils from the analysis. One camera in the scented latrine treatment malfunctioned during 1 week of the manipulation period. To remedy this, we replaced the missing visit and mark data for that week first with the average number of visits and scent marks per week during the manipulation period. To ensure that replacing the missing values with the averages did not

bias the results, we also ran the models with the missing data replaced with a zero for that week and compared the results. We used a multi-level (nested) mixed effects regression model to examine the effects of time (pre, manipulation and post), scent treatment (scented vs control), and their interaction on the duration of wild resident devil latrine visitation, investigation, and marking behavior. We then used the same type of model to examine the effect of scent manipulation on releasee devil behavior at latrines following release (post-manipulation period) as this was the only period in which they were present in the landscape. We then conducted a series of a priori tests within and between the different latrine treatment periods to examine our predictions regarding discrimination and temporal behavioral changes resulting from our experimental manipulations.

Results

Experiment 1: Effect of social familiarity on odor discrimination in ex situ devils

Results from our ex situ experiment indicate that both familiarity and the sex of the scent donor influence devil response to odor cues. There was a main effect of donor sex and familiarity on time spent investigating the feces (sex: $\chi^2 = 8.83$, $N = 12$, $p < 0.01$; familiarity: $\chi^2 = 9.99$, $N = 12$, $p < 0.01$; Fig. 3), and a significant interaction effect between familiarity and donor sex on time spent investigating the feces ($\chi^2 = 4.89$, $N = 12$, $p < 0.03$). Devils spent more time investigating unfamiliar donor feces, but the effect was strongest when the donors were female. Devils spent significantly more time investigating the feces of novel female donors than familiar ones. Finally, there was no interaction between subject sex and donor sex on feces investigation ($\chi^2 = 2.19$, $N = 12$, $p = 0.14$). Devils spent more time scent marking in response to unfamiliar conspecific feces ($\chi^2 = 6.90$, $N = 12$, $p < 0.01$; Fig. 3) regardless of donor sex ($\chi^2 = 0.77$, $N = 12$, $p < 0.38$), and there was no interaction between subject and donor sex in time spent scent marking ($\chi^2 = 0.10$, $N = 12$, $p = 0.75$). Feces age (time in freezer) did not influence devil investigation or scent marking behavior (investigate: $z = 0.06$, $N = 12$, $p = 0.95$; scent marking: $z = 1.06$, $N = 12$, $p = 0.29$).

### Field survey: Wild latrine habitat selection

We identified 18 devil latrines on our transects (Fig. 1). In all but one case, Tasmanian devil latrines were located in transect plots identified as ecotone habitat (Fisher's exact test: $p < 0.01$). Twelve of the 18 latrines found were along game trials, walking paths, and roads. Using AIC backwards selection, the only habitat variable that was predictive of latrine sites within ecotone was the presence of shrubs, and it was perfectly predictive.

Experiment 2: Effects of pre-release scent familiarization on the behavior of wild residents and releasees

We analyzed the frequency of devil visits, frequency of visits that included scent marking, and also the duration of behaviors that occurred during each visit quantified from camera trap videos. Based on the hypothesis that devils become familiar with conspecifics through scent exposure, we predicted that the frequency of olfactory-related behavior of wild resident devils would initially increase at experimental latrines where we had placed the scent of novel individuals relative to control latrines. This prediction was supported by both the frequency and duration data. Using the average frequency of visits and marks per week to replace the missing data, results indicate that wild devils visited ($z = 3.17$, $p < 0.01$) and scent marked ($z = 3.19$, $p < 0.001$) at experimental latrines more than control latrines during the manipulation period (Fig. 4). The results did not change when zeros were used to replace the missing data (visit: $z = 2.34$, $p < 0.02$; scent marks; $z = 3.21$, $p < 0.001$).

The amount of time wild resident devils visited latrines and spent investigating scent within visits provided additional support of this prediction. We found a significant interaction between scent treatment and time spent visiting ($\chi^2 = 9.93$, $p < 0.01$) and investigating ($\chi^2 = 14.30$, $p < 0.01$) latrines (Fig. 5). Furthermore, wild resident devils spent significantly more time visiting ($z = 3.17$, $p < 0.01$) and investigating ($z = 4.69$, $p < 0.01$) scent at experimental latrines than control latrines during the manipulation period, but not in the pre-manipulation period (visit: $z = -0.44$, $p = 0.66$; investigate: $z = -0.42$, $p = 0.68$). Examining how devils' behavior changed following introduction of experimental scent, we found that wild resident devils also increased visitation ($z = 2.19$, $p = 0.03$) and investigation ($z = 4.21$, $p < 0.01$), between the pre-manipulation and manipulation periods at the experimental latrines, while at the control latrines there was a decrease in visitation ($z = -2.25$, $p = 0.02$) and no change in the duration of investigation ($z = -1.43$, $p = 0.15$). Next, we examined wild resident devil behavior during post-manipulation, when the releasees were likely depositing scent at both experimental and control latrines. We expected to see a decline in visitation, investigation, and marking at the experimental latrines as resident devils became habituated to the scent of releasees, and that was indeed the case. Between the manipulation period and the post-manipulation period, visitation and investigation duration decreased at experimental latrines (visitation: $z = -4.00$, $p < 0.01$; investigation $z = -4.36$, $p < 0.01$; Fig. 5), but remained the same at control sites (visitation: $z = -1.18$, $p = 0.24$; investigation $z = -1.13$, $p = 0.26$). Finally, our results indicate that resident devil visitation and investigation did not differ

Fig. 3 Time spent investigating (s) and marking (s) by male and female subjects in response to familiar (solid bars) and unfamiliar (hatched bars) donors of each sex. Error bars represent SE. Created in R package ggplot2 (R version 4.2.2; R Development Core Team 2023)
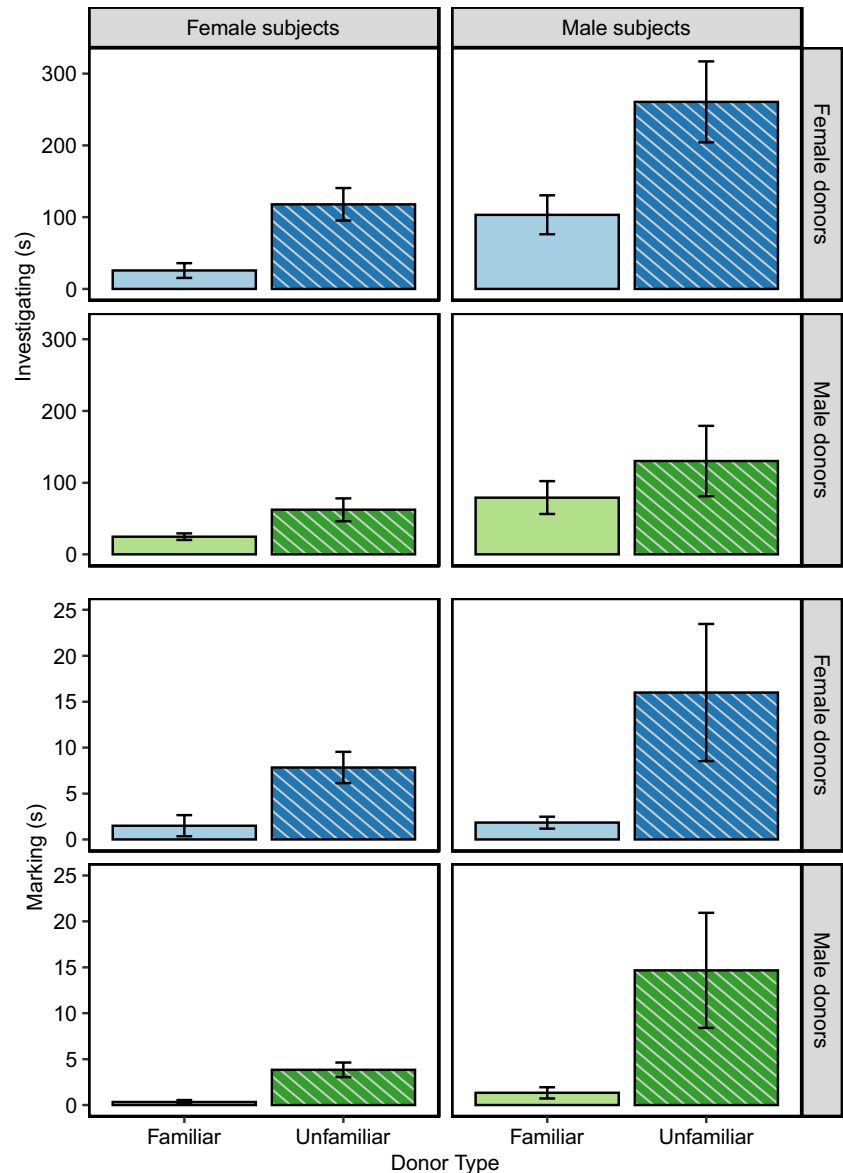
between control and experimental latrines during the post-manipulation period (visitation: $z = -1.18$, $p = 0.24$; investigation $z = -1.13$, $p = 0.26$). With regard to scent marking, overall, wild resident devils spent more time scent marking in experimentally manipulated than in control latrines ($\chi^2 = 4.07$, $p = 0.04$), but there was no significant interaction between time and treatment ($\chi^2 = 3.62$, $p = 0.16$).

We evaluated the behavior of newly released ex situ devils upon release. The experimental manipulation increased visitation and marking by resident devils (Figs. 4, 5); and thus, the experimental latrines presumably contained greater quantities of fresh resident scent than control latrines. Combined with the presence of their own odors or the odors of their prior enclosure mates, we expected experimental latrines to attract more interest and predicted that released devils would show higher levels of visitation or olfactory communication

behavior at experimental than control latrines. Our results support this prediction (Fig. 6). While newly released devils spent the same amount of time visiting and marking latrines regardless of experimental scent manipulation (visit: $z = 2.92$, $p = 0.09$; mark: $z = 0.04$, $p = 0.83$), they spent significantly more time investigating experimental latrines compared to controls ($z = 7.36$, $p < 0.01$).

Discussion

Our findings from both in situ and ex situ environments indicate the importance of odor familiarity and latrines as communication centers in the behavioral ecology of the Tasmanian devil. Clearly, devils do not defecate at random on the landscape and instead select communal

Fig. 4 Boxplots showing resident wild devil visit and marking frequency in the pre-manipulation and manipulation periods. Thick horizontal lines represent the median, vertical lines depict the smallest and largest values, within 1.5 inter-quartile range, and circles represent outside values. Created in R package ggplot2 (R version 4.2.2; R Development Core Team 2023). Photo credit San Diego Zoo Wildlife Alliance
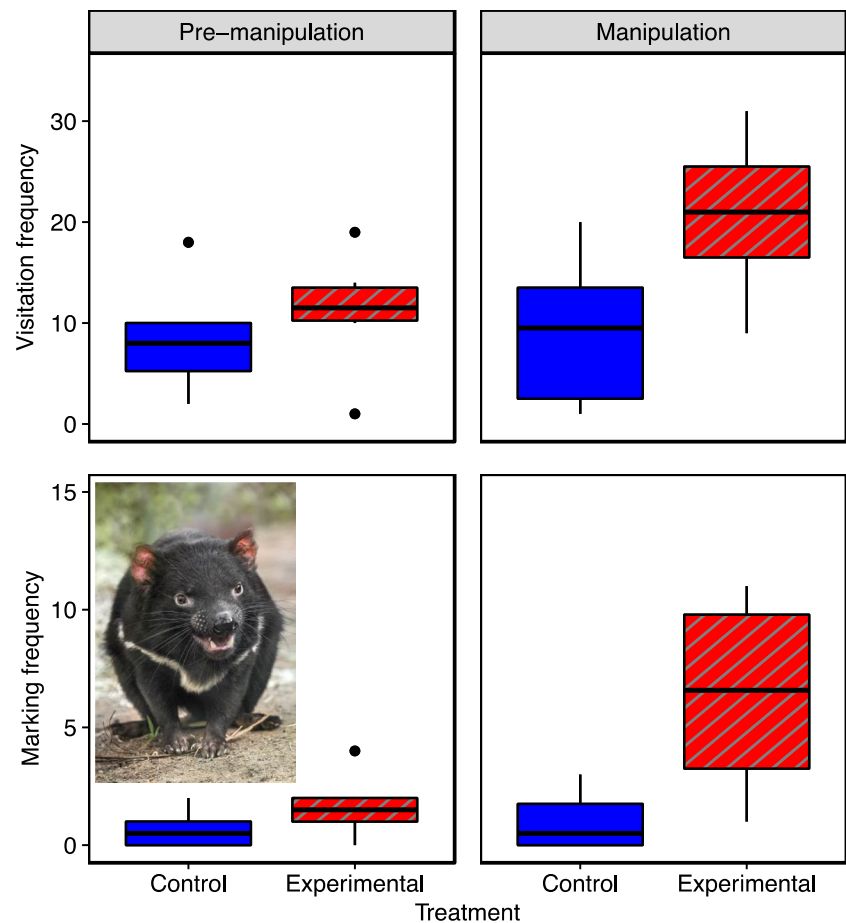

sites for defecation and marking at conspicuous sites at transitions between vegetation types and along established animal trails and roads. The presence of shrubs at all documented latrine sites is plausibly indicative of selection for prominent visual features to signal the presence of the latrine site to conspecifics. We propose that these selective scent deposition behaviors serve to maximize the probability that other devils will encounter scent signals, making latrines a more effective means of communication. These findings are consistent with findings for a variety of species that use prominent features and landmarks that incorporate a visual component to scent signals, such as trail crossings (Zollner et al. 1996; Attum et al. 2006; Barja and List 2006; Balestrieri et al. 2009; Torgerson 2014).

Placement of latrines with regard to home range also varies with function, with some species depositing scent on range peripheries (Brashares and Arcese 1999; Stewart et al. 2001; Wronski et al. 2006) and others selectively marking in the range core (Gorman and Mills 1984; Jordan et al. 2007; Wronski et al. 2013; Eppley et al. 2016). When territoriality is economically defensible, scent marks and latrines are typically located along borders to advertise territory ownership to neighbors and intruders. Species

with larger home ranges cannot reliably mark boundaries and thus intruders may enter the range without encountering marks; these species typically mark the core of their ranges where eventually intruders will encounter them and be able to assess territory ownership/range occupation. Devils occupy large extensively overlapping home ranges (Pemberton 1990), utilizing anthropogenic linear features such as fence lines and roads for movement and the pasture/cover interface for foraging (Andersen 2017). Thus, placement of latrines in these areas likely maximizes efficacy of scent communication and supports the notion that devils are not simply solitary in the classic sense, and that they use latrines to coordinate a variety of social functions that remain unknown.

Our study is the first to publish quantitative data on the Tasmanian devil response to conspecific odors, and we have shown that odor familiarity governs the nature of this response. Ex situ managed devils showed a strong preference to investigate unfamiliar odors, an effect that was strongest when devils were investigating female odors. For males, an unfamiliar female may represent a new reproductive opportunity, and hence the heightened interest. For females, interest in male odors may be more pronounced when they are physiologically prepared to mate (estrus), but

Fig. 5 Duration (s) of visitation, investigation, and marking across time in control (solid bars) and experimental (hatched bars) latrines by wild resident devils. Error bars represent SE. Created in R package ggplot2 (R version 4.2.2; R Development Core Team 2023)
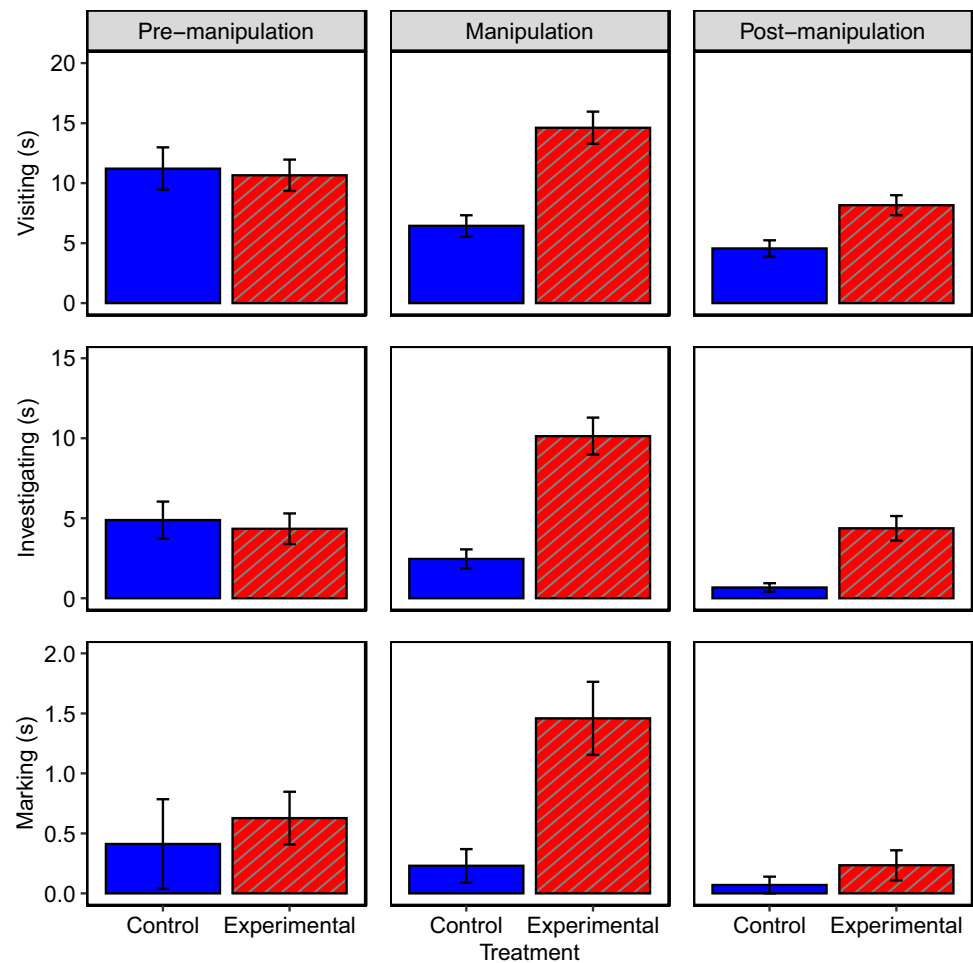
it may be possible that estrous females would prefer odors of familiar males at that time, as has been found for other species (Fisher et al. 2003; Roberts and Gosling 2004). For female devils investigating female odors, perhaps heightened investigation of unfamiliar female odors represents the potential threat of range incursion by an unfamiliar female. By contrast, unfamiliar odors increased marking behavior similarly regardless of donor or receiver sex, indicating motivational priority to increase attempts to communicate with all unfamiliar individuals.

Furthermore, wild resident devils visited latrines more often, marked more, and spent more time investigating feces when we experimentally placed feces from unfamiliar ex situ managed animals in latrines. Using both spatial and temporal controls, we demonstrated that resident devils invest more in visiting and communicating via scent deposition when they encountered scent from unfamiliar individuals. With time and repeated exposure, resident devils showed less interest in the odors of unfamiliar individuals, presumably because they have become more familiar with their scent. Following the end of our scent manipulations in latrines and the release

of now familiar-smelling devils, resident devils did not increase their latrine visitation and olfactory communication behaviors. Alternatively, it is possible that devils simply responded to the additional quantity of scent at experimental latrines. However, several lines of evidence suggest that this is unlikely. First, our findings in experiment 1 show the same pattern of heightened interest to unfamiliar scent. Taken together with unpublished data that show a decline in response with repeated exposure (habituation) to a conspecific devil's scent (DMS et al., unpublished data), these results suggest that the same mechanism (familiarity) is operating in experiment 2. Moreover, the response to experimental latrines declined in the post-manipulation phase even while the quantity of feces did not decline.

Upon release of ex situ managed devils that served as scent donors, resident devils responded less to manipulated latrines, suggesting that they now classified odors from these animals as familiar. Interestingly, this familiarization effect appeared to extend to unmanipulated latrines, as there was no increase in resident devil interest in control latrines during the post-manipulation period despite
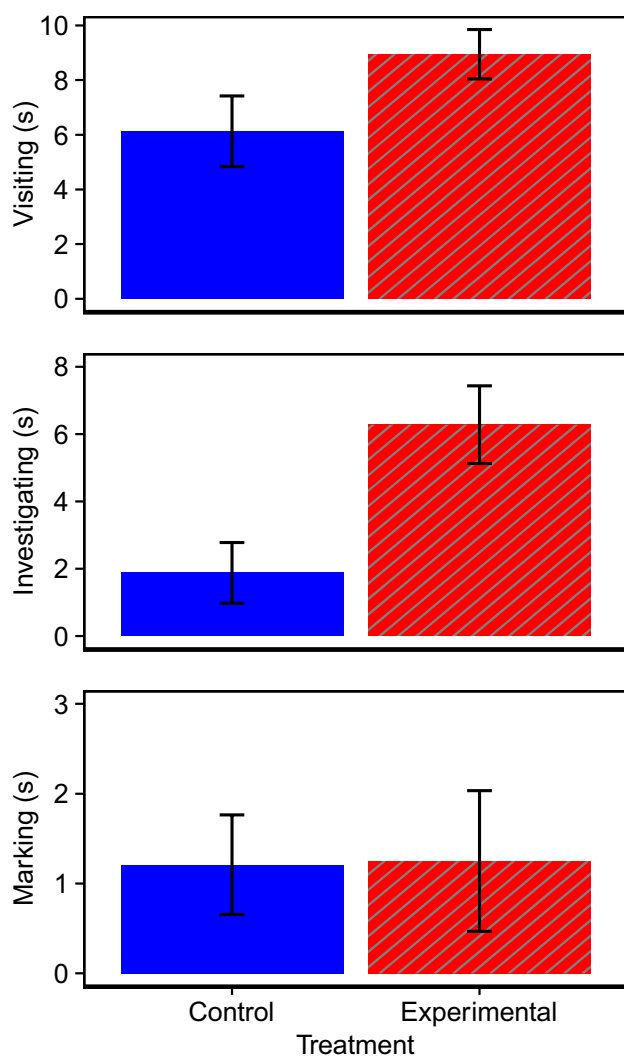
Fig. 6 Duration(s) of visitation, investigation, and marking in control (solid bars) and experimental (hatched bars) treatments by ex situ managed devils following release to the wild. Error bars represent SE. Created in R package ggplot2 (R version 4.2.2; R Development Core Team 2023)

the release of ex situ managed devils in the landscape. By contrast, released devils spent more time investigating feces at manipulated latrines containing their own scent and that of their enclosure mates in the ex situ facilities. Since released animals have not yet settled into established home ranges—unlike residents or devils in our ex situ trials—this response is unsurprising. Released devils may have increased interest in familiar odors because they represent familiar animals in a landscape occupied mostly by unfamiliar, threatening individuals. We do not, however, know if encountering familiar scent reduced stress, facilitated social cohesion with members of the release group, or reduced dispersal away from the release site, but all of these scenarios are plausible outcomes. Alternatively,

released devils may have investigated odors at manipulated latrines because they had been visited more often and more recently by resident devils investigating the experimentally placed scent.

Because the release area was small relative to devil ranging patterns, we were unable to conduct an experiment with some resident devils pre-exposed to scent of ex situ managed devils before release while devils living in other areas served as controls with no pre-exposure to the odors of translocated conspecifics. This is unfortunate, but often to be expected in a conservation context with an endangered species where experimental manipulation can prove difficult. However, we expect that our efforts to increase odor familiarity by exposing wild devils to the odors of ex situ managed devils and vice versa before release did serve to reduce aggression between residents and translocated devils following release. The fact that interest by resident devils in the experimental latrines diminished following release of familiar-smelling devils suggests habituation and familiarity with the odor of those individuals. In other species, odor familiarity has been shown to reduce aggression in direct encounters (López and Martín 2002; Rosell and Bjørkøyli 2002; Nakamura et al. 2007; Zenuto 2010), and we have reason to believe this is a plausible outcome for devils. Ex situ managed devils also demonstrated greater olfactory interest in unfamiliar animals in experimental trials, again emphasizing that devils may perceive unfamiliar individuals to represent a greater threat to residents.

We view these experiments as part of a larger adaptive management program to wed science to management a priori by establishing agreed-upon research priorities with scientists and managers working in concert to plan and implement research that will better inform management (Hogg et al. 2017; Shier et al. 2019a). In these circumstances, the perfect experiment is not always possible as there is inevitable tension between research and management goals, yet the outcome of research should reduce uncertainty about the outcomes of management actions. Although we have not demonstrated that pre-exposure to odors of devils reduces social aggression, dampens dispersal, and increases survival in translocations, our results are consistent with this hypothesis, and therefore allow us to place higher priority on these management actions in the future. We know from other species that efforts to dampen dispersal and minimize social disruption improve translocation outcomes (Shier 2006; Shier and Swaisgood 2012; Poirier and Festa-Bianchet 2018; Swaisgood and Ruiz-Miranda 2019), and we now have evidence suggesting odor familiarity may be a useful tool to apply towards these ends in the Tasmanian devil. Moreover, if scent is used for management translocations to familiarize resident devils and release cohorts, our results suggest that seeding a subset of latrines can have a landscape level effect in this wide-ranging species.

We encourage future research on devils and other species reliant on chemical communication to conduct experiments manipulating odor familiarity to determine its value in improving the science of translocation biology and use of rewilding as a management tool. In addition to this tool, latrine distribution size and use dynamics should be explored as a population monitoring tool (Tuyttens et al. 2001) or to monitor establishment of released animals (Wronski and Plath 2010). Latrine use also has additional implications for biodiversity by virtue of its impacts on vegetation structure and composition (Ben-David et al. 2005; González-Zamora et al. 2012). Latrine use may also be problematic if poachers use animals' predictable visitation to specific locations to increase harvest rates (Attum et al. 2006). Latrine ecology's application for conservation is only just beginning to be explored, but is a promising avenue for future conservation interventions. Applications of chemical communication include influencing habitat selection, population monitoring, deterring predators, facilitating successful translocation, and curtailing the movement of predator species to reduce human-wildlife conflict (Campbell-Palmer and Rosell 2011).

Declarations

Ethics approval All work was conducted under the permission of the ethical committee for animal research of the Department of Primary Industries, Parks, Water and Environment (DIPIPWE), and standard operating procedures for animal husbandry, handling, and health checks.

Competing interests The authors declare no competing interests.

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