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The Behavioral Significance of Olfactory Scent Cues in the Tasmanian Devil

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science
in Biology

by

Elizabeth Ellen Reid-Wainscoat

2018

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ABSTRACT OF THE THESIS

The Behavioral Significance of Olfactory Scent Cues in the Tasmanian Devil

by

Elizabeth Ellen Reid-Wainscoat

Master of Science in Biology

University of California, Los Angeles, 2018

Professor Gregory F. Grether, Chair

The Tasmanian devil (*Sarcophilus harrisi*) is a nocturnal carnivorous marsupial that has suffered precipitous decline in the past 20 years due to a contagious fatal cancer. To provide the best management practices to help re-establish wild populations it is crucial to understand the behavioral ecology of this species. Initial studies proved that, despite their classification as a solitary, non-territorial species, olfactory communication plays a significant role in Tasmanian devil social structure and scent cues deposited at shared latrine sites provide important social cues. To better understand the role of the latrine sites, we tested whether Tasmanian devils communicate sex, season and individual differences through scent cues. Specifically, we analyzed the chemical composition of anal scent gland secretions using gas chromatography-mass spectrometry, which revealed significant differences between seasons (breeding/non-breeding), sexes, and individual males. To determine if conspecifics could perceive these differences, we presented feces to captive devils and found that the devils can habituate to a conspecific's feces after repeated presentations and distinguish it from that of a novel conspecific, as indicated by significantly higher rates of investigation. This proves to also be true for familiar conspecifics as compared to unfamiliar conspecifics. This is the first documentation of specific semio-chemical information in Tasmanian devil scent cues and suggests evidence of sociality previously not described for this species. Application of these results can be used to improve captive breeding and reintroduction efforts for this endangered species and inform management strategies for other carnivore restoration efforts.

The thesis of Elizabeth Ellen Reid-Wainscoat is approved.

Peter Nicholas Nonacs

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University of California, Los Angeles

2018

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Introduction

Scent Ecology in Mammals

Chemical signals in mammals derive from mixtures of volatile and non-volatile compounds expressed in urine, feces, saliva, breath, tears, sweat, milk, amniotic fluid, genital secretions, and specialized glandular secretions, that underlie a complex mode of communication. Such signals dictate many behavioral interactions both at the intra- and interspecific level (Eisenberg and Kleiman, 1972; Gosling and Roberts, 2001; Ralls, 1971). Specifically, they are used by many mammalian species to communicate species, age, sex, reproductive condition, dominance rank and health status. They can help reinforce social hierarchies within groups, define territories between individuals or groups, assist with mate selection and act as alarm cues (Ralls, 1971). They can also be manipulated to convey false cues and thus act as a chemical lure for prey species (Soso et al. 2014). In addition, some species' chemical cues are complex enough to convey individual identity.

Individual odors used in recognition have been documented in a wide variety of carnivores including African dwarf mongooses (*Helogale undulata rufus*) (Rasa, 1973) the small Indian mongoose (*Herpestes auropunctatus*) (Gorman, 1976), mice (*Mus musculus*) (Bowers and Alexander, 1967), giant pandas (*Ailuropoda melanoleuca*) (Swaigood et al., 1999), wolves (*Canis lupus*) and dogs (Brown and Johnston, 1983). Such discrimination between individuals is a critical component of species social structure and can help maintain dominance hierarchies, mating pairs, extended parent-offspring interactions, and ingroup-outgroup associations (Buesching et al., 2002; Harrington, 1981; Woodley and Baum, 2003).

Individual discrimination and its applications have been associated with increased fitness (Johnstone and Dugatkin, 2000; Tibbetts and Dale, 2007). In social species this type of discrimination is beneficial when their response to an individual may depend on the degree of relatedness or their social value (Axelrod and Hamilton, 1981; Barnard and Burk, 1979;

Hamilton, 1964), as has been demonstrated in several species (Cheney and Seyfarth, 1986; Connor et al., 1992; East et al., 1993; Mateo, 2004). Individual discrimination can also aid in the identification of conspecifics that may entail costs such as sexual harassment (Clutton-Brock and Parker, 1995) or infanticide (Hausfater and Hrdy 1984) to ensure that these individuals are avoided. The potential application of this discrimination could be used to recognize individuals and to predict their likely response. Such an ability would be an important social skill that might confer fitness benefits (Axelrod and Hamilton, 1981; Johnstone and Dugatkin, 2000; Mateo, 2004; Tibbetts and Dale, 2007).

The placement of these olfactory signals in the environment is also important when determining their behavioral significance. Placement of these scent cues on objects in a species' habitat is called scent marking (Gosling and Roberts, 2001). Scent marks are placed often in the absence of the receiver and may only be detected after the signaler has left the area (Gosling and Roberts, 2001). This delay in detection, unlike other communication cues such as vocalizations, allows for conspecifics to perceive the presence of the signaler long after the signaler has left the area. Over time signal degradation does occur as the marks are exposed to the environment (e.g. rain; Alberts, 1992), but this can be counteracted by repeated marking. Male mammals generally will re-mark active scent marks such that hundreds of marks may be regularly replenished. Similar to other forms of olfactory signaling where odor signals are directly transmitted from signaler to receiver, scent marks in mammals are used in a variety of contexts from mate receptivity and selection to territorial defense, dominance status and health. In species with dominance hierarchies scent marking can aid in the establishment and maintenance of territories (Jedrzejewski et al., 2003).

A special case of scent marking is the use of latrine sites. Latrines are places where animals habitually defecate and urinate. When numerous individuals utilize a single latrine site to scent mark and counter mark using urine, glandular secretions or feces, the sites are

classified as “composite latrines” (Macdonald, 1980). Composite latrines sites have been documented in a wide variety of mammalian taxa including primates (*Lepilemur sp.* and *Hapalemur sp.*; Irwin et al., 2004) ungulates (Estes, 1991; Leuthold, 1977), rodents (*Arvicola terrestris*; Woodroffe et al., 1990), lagomorphs (*Oryctolagus cuniculus*: Sneddon, 1991), and marsupials (*Dasyurus maculatus*: Kruuk and Jarman, 1995) and at least 34 species in the order Carnivora representing 7 families (Canidae: Macdonald, 1979); (Felidae: Moreno and Giacalone, 2006); (Herpestidae: Jordan et al., 2011); (Hyaenidae: Kruuk, 1972); (Mustelidae: Zhou et al., 2015); (Procyonidae: Barja and List, 2006); (Viverridae: Forest et al., 2008).

Extensive research on the type, quantity and composition of cues deposited at composite latrines as well as the behaviors exhibited during investigation and deposition has allowed researchers to better understand the significance of these sites. The most common hypotheses to explain the function of these communal latrines include those pertaining to resource ownership including territorial boundaries, feeding sites, breeding and/or sleeping sites, and mate acquisition/defense. Other hypotheses have described the potential for latrine sites to act as information centers communicating reproductive advertisement and orientation. Alternatively, they could have direct health and fitness benefits including reduced parasite loads by avoiding mates that unintentionally signal disease (Mitchell et al., 2017) or detection of predator presence by prey species utilizing the territorial marks of their predator species (Russell and Banks, 2007). Large communal latrines that are found at path intersections and at the center of several individual’s home ranges indicate that their function is primarily informational. Small latrines spaced around the perimeter of an individual’s or group’s territory support the resource defense hypothesis.

Application of Scent Ecology to Improve Release Outcomes

Understanding a species’ behavioral ecology and the function of scent cues has become increasingly important to wildlife managers and conservation scientists specifically in

the context of translocations and reintroductions. Species restoration efforts across a variety of taxa have proven to be difficult, with many failing to re-establish healthy wild populations (Kleiman et al., 1994; Maynard et al., 1995; Miller et al., 1994; Wolf et al., 1998).

Specifically, post-release dispersal is often associated with high mortality rates during the first days and weeks following release (Short et al., 1992; Wolf et al., 1998). Dispersal can increase the risk of predation (Daly and Paton, 1990) and the risk of animals entering human inhabited areas, where vehicle strikes increase mortality rates, as seen in the Tasmanian devil (Grueber et al., 2017).

Post-release dispersal could be influenced by unfamiliarity with individuals within their release cohort (Shier, 2006) or by resource competition with conspecifics (Maynard et al., 1976) at the release site. Alternatively, it could be the absence of conspecifics at the site that drives individuals away (conspecific attraction; Stamps, 1988) or the lack of familiarity with the habitat at the release site, especially if it differs from their natal habitat (natal habitat preference induction; Davis and Stamps, 2004; Stamps and Swaisgood, 2007).

To combat these issues, conservation biologists have begun to utilize controlled experiments to assess the impact of different release strategies on dispersal and survival rates (Griffiths and Pavajeau, 2008). The first techniques that were trialed simply provided a more gradual transition from a captive environment to the wild. These techniques included supplemental food and human engineered shelters as well as acclimation pens at the release site. These efforts were meant to encourage settlement and provide critical resources during the first few days to week in the wild (Swaisgood, 2007). Despite the effectiveness of these techniques, there were still unresolved issues that stemmed from a misunderstanding or lack of consideration for the behavioral ecology of each species (Swaisgood, 2007).

Understanding the behavioral ecology of the species and the impact that reintroductions and translocations have on the social system has proven integral to the

success of such efforts (Stamps and Swaisgood, 2007). To gain a better understanding of these impacts, researchers have begun to manipulate behavioral cues that could be used to signal quality habitat and thus encourage site fidelity after release (reviewed in Wolf et al., 1998). Such cues include model decoys (Jeffries and Brunton, 2001), song playbacks (Ward and Schlossberg, 2004) and white paint to mimic feces in a breeding colony (Sarrazin et al., 1996).

In addition to increasing familiarity with the new habitat, it is also important to familiarize releasees with each other and with the resident conspecifics that live in the immediate area surrounding the proposed release site (Shier, 2006; Stamps and Swaisgood, 2007). Initial research has revealed that for both social and solitary species, familiarization between individuals in the release cohort can significantly increase fitness post-release. For example, black-tailed prairie dogs (*Cynomys ludovicianus*) translocated within family groups were five times more likely to survive and had significantly higher reproductive success than those translocated without families (Shier, 2006). Likewise, kangaroo rats (*Dipodomys*) translocated with neighbors traveled shorter distances before establishing territories, had higher survival rates, and had significantly higher reproductive success than kangaroo rats translocated without neighbors (Shier and Swaisgood, 2012).

Tasmanian Devil Scent Ecology

The application of behavioral ecology to species restoration efforts have advanced the field of conservation biology and increased the possible management strategies that can be applied to help restore populations of critically endangered species. One such mammal is the Tasmanian devil (*Sarcophilus harrisii*), which has declined significantly in numbers over the past 20 years due to a contagious fatal cancer: Devil Facial Tumor Disease (McCallum et al., 2007). While the species was common and considered stable two decades ago, it is now

considered endangered at both a national (Environment Protection and Biodiversity Conservation Act 1999) and state level (Threatened Species Protection Act 1995).

The Tasmanian devil (*Sarcophilus harrisi*) represents the largest extant carnivorous marsupial present today and is the primary scavenger for the macropod carrion that exists at high levels throughout the island state. Despite their critical role as a keystone predator and scavenger, to date, devil behavioral ecology has not been extensively studied. However, the anecdotal evidence that exists from managers and husbandry staff suggest that Tasmanian devils have a rudimentary social system that could be utilizing chemical cues present in their feces. Tasmanian devils are considered nocturnal and solitary, but not territorial (Pemberton, 1990). Their home ranges are large and overlap significantly with other conspecifics (Shier and Fox, 2017). Although direct interaction is rare, conspecifics are known to interact aggressively around prey carcasses and during the mating season where males will bite and drag females into dens (Hamede et al., 2008; Pemberton and Renouf, 1993). Chemical communication is assumed to take place at latrines that are present along common travelling routes. These latrines are comprised of feces from multiple individuals at most of these sites (Jones, 1998; Owen and Pemberton, 2005). Direct observations of scent marking behavior has been documented in captivity where individuals often deposit feces and anal scent secretions around their enclosures (Stephen Izzard, DPIPWE, pers. comm).

Other large mammals have been observed depositing anal scent secretions by wiping their protruded anal gland over objects, typically grass stalks, thereby creating a 'scent post' (Burgener et al., 2009). Scent marking with the anal gland is termed 'pasting' (Kruuk, 1972). Frequent scent-mark in latrines and these scent posts are investigated by other group members, which may also paste their own scent on or near existing scent marks, a behavior termed 'overpasting' (Burgener et al., 2009; Hofer et al., 2001). If, as has been suggested (Hofer et al., 2001), one function of depositing scent at social centers is to create a long-

lasting olfactory signal of an individual's continued membership of the group, anal gland secretions would be expected to contain individual identity cues.

In captivity, devils have more opportunities to interact with conspecifics due to smaller enclosures and controlled mating interactions as compared to their typical home ranges in the wild. Conspecifics that are unfamiliar often scent mark, posture and vocalize with bluff charges and open mouth biting during their first few minutes of interaction. Devil husbandry staff have observed these interactions lasting up to 24 hours and have documented such interactions escalating to actual physical attacks with sustained injuries. One trend observed was that conspecifics that are familiar with each other tend to have less aggressive interactions when physically introduced and settle more quickly into a dominance hierarchy (Stephen Izzard, DPIPWE, pers. comm). This phenomenon, referred to as the 'dear enemy' effect, has been documented in other mammal species such as the Eurasian beaver (*Castor fiber*) (Rosell and Bjorkoyli, 2002), but is often associated with territorial species.

This evidence suggests a level of olfactory socialization, but the specifics of what is being communicated through these scent cues and how it impacts their social structure in the wild is still not well understood. To better understand the behavioral significance of these chemical cues within the context of Tasmanian devil sociality, we quantified the chemical compounds present in Tasmanian devil anal scent glands to determine differences in composition and amount between seasons, sex, and individuals. If the anal gland secretions of Tasmanian devils contain information about individual identity, then conspecifics would be expected to be able to differentiate between familiar and unfamiliar individuals and spend more time investigating novel conspecifics. To test this hypothesis, we measured whether captive Tasmanian devils are capable of habituating to a conspecific through repeated presentations of feces and discriminating between novel and familiar individuals' feces.

Methods – Part I

Study Population

This study was conducted on Tasmanian devils located in a captive-breeding center in Cressy, Tasmania run by the Save the Tasmanian Devil Program. Data was collected from April 2015 – April 2017. All subjects were adults (> 1 year old) housed in individual enclosures to ensure accurate sampling. The Captive Research Advisory Group (CRAGS) gave approval for use of all animals in the captive breeding program. Animal Ethics approval was acquired through the DPIPWE committee.

Scent Gland Secretion Collection

Anal scent gland secretions were obtained from males and females during routine veterinary check-ups in DPIPWE-run captive breeding centers (containing approx. 150 animals). A minimum of 2 samples were taken from each subject at separate times to account for individual variability. Samples were collected by gently massaging the anal gland region while a cleaned glass vial covered the gland duct. Approximately 0.3ml of secretion was collected from each animal. All samples were then stored prior to analysis in a -80°C freezer before being transported to the University of New South Wales (UNSW) on dry ice and then stored in another -80°C freezer until analysis was conducted.

Chemical Analysis

Solid phase microextraction (SPME) is a gas chromatography–mass spectrometry (GC-MS) technique that has successfully been used to analyze volatile compounds from mammalian scent-marks (Vas and Vékey 2004; Wood et al. 2009; Soso et al. 2014; Robley 2015). SPME GC-MS methods for this study were derived from Carthey (2013). Prepared GC-MS samples (N = 78) were made up to approximately two grams (mean weight of GC-MS samples = 1.88 g) using metal spatulas; which were cleaned only with tap-water to keep

cleaning consistent and avoid detergent contamination. Samples were then placed in headspace caps that had been previously weighed and were then re-weighed to get exact weight of each sample. All sample runs were conducted with two periodic blanks using the same preparation to counter background peaks that are commonly present in SPME analysis.

Statistical Analysis

Statistical analyses for this study were conducted in R-Studio v1.0.153 (R-Core Team, 2017). We utilized two different methods of sample processing. Initially, 3 replicates were run for 9 individuals using a Simple scan method to identify potential compound peaks in each sample. The raw GC-MS outputs from these samples were pre-processed using AMDIS v2.70 (Smart et al. 2010). The NIST W9N11 v2.0 Library database was then used to search for each compound (N compounds = 800,000+). A minimum match factor of 70 was used and all components above the 0.0% threshold were searched for. The instrument m/z limits directed the search to select from all components and consider all models. These options gave the search a broad scope and allowed a vast amount of compound identities to be produced. Resulting identities were so numerous that a single sample needed to be pre-processed twice, since the maximum number of identities possible per search (N = 10,000) was consistently reached in the first scan.

These data were then used to create a target list of 90 chemical compounds found in the majority of test samples. This target list was based on the retention times and principle ions of these compounds. These ions were chosen because of their relative ion abundances, high m/z values, and lack of interference from other Tasmanian devil components. This method is known as the selective ion monitoring (SIM) mode. SIM detects specific analytes with enhanced sensitivities relative to the full-scan mode. The sensitivity of SIM GC/MS can be increased by a factor of 10 to 100 times that of Full-Scan GC/MS. This allowed a comparison of not only the presence/absence of compounds, but also a comparison of the

relative abundance of each compound between samples. This method was applied to all samples (N=78) including a technical replicate for each.

Prior to statistical analysis, chemical compound data was arranged in two data-frames and then merged so that the % total ion count (TIC) corresponded to the correct cell in the full compound list, for each Tasmanian devil sample. A nominal value of 1.0×10^{-9} was input for those data cells that contained no %TIC value and implemented a log transformation on the resultant matrix (Jordan et al. 2016). The nominal value ensured future analyses ran correctly since it was magnitudes below the smallest %TIC value, preventing zero and near-zero variance (Apps 2013); while the transformation centered and scaled the data (Jordan et al. 2016). In preparation for the discriminant function analysis (DFA), all compounds that only occurred once were eliminated, as these could not logically be involved in coding demographic categories (Jordan et al. 2016). The compound name rows that were not filtered by the 3-blank protocol were then melded and the final compound list was transposed.

Multivariate chemical data in this study required dimensionality reduction through principal components analysis (PCA) (Jordan et al. 2016) plotted utilizing ggplot2 (Wickham, 2016). This reduction produced a PCA score matrix which was input into a discriminate function analysis (DFA) (Jordan et al. 2010) modelled through MASS (Venables and Ripley, 2013) and plotted in ggbiplot (Vu, 2011) . The prior probabilities of each sample grouping were then calculated to produce the predicted group assignment. The predictive model was bootstrapped, calculating the number of iterations (from a total of 10000) that performed better than the classification. This was then used to produce a p-value score for each DFA model. For example, if 50/10000 iterations had better percentage classification than ones that were generated, then $p=0.005$.

Results – Part I

Chemical Analysis

The discriminate functions analysis showed that Tasmanian devil anal scent gland secretions proved to be individually distinct for males (Male ($N=10$) – figure 1a, 1b, 95.8% group discrimination prediction, $P < 0.0001$). When compared together, male and female anal scent gland samples collected during breeding season contained sexually specific information (Sex Demographic ($N=10$) – figure 1c, 1d, 80% group discrimination prediction, $P < 0.0001$). In addition, there was a significant effect of seasonality (breeding versus non-breeding) for males (Season Demographic ($N=10$) – figure 1e, 1f 99% group discrimination prediction, $P < 0.0001$).

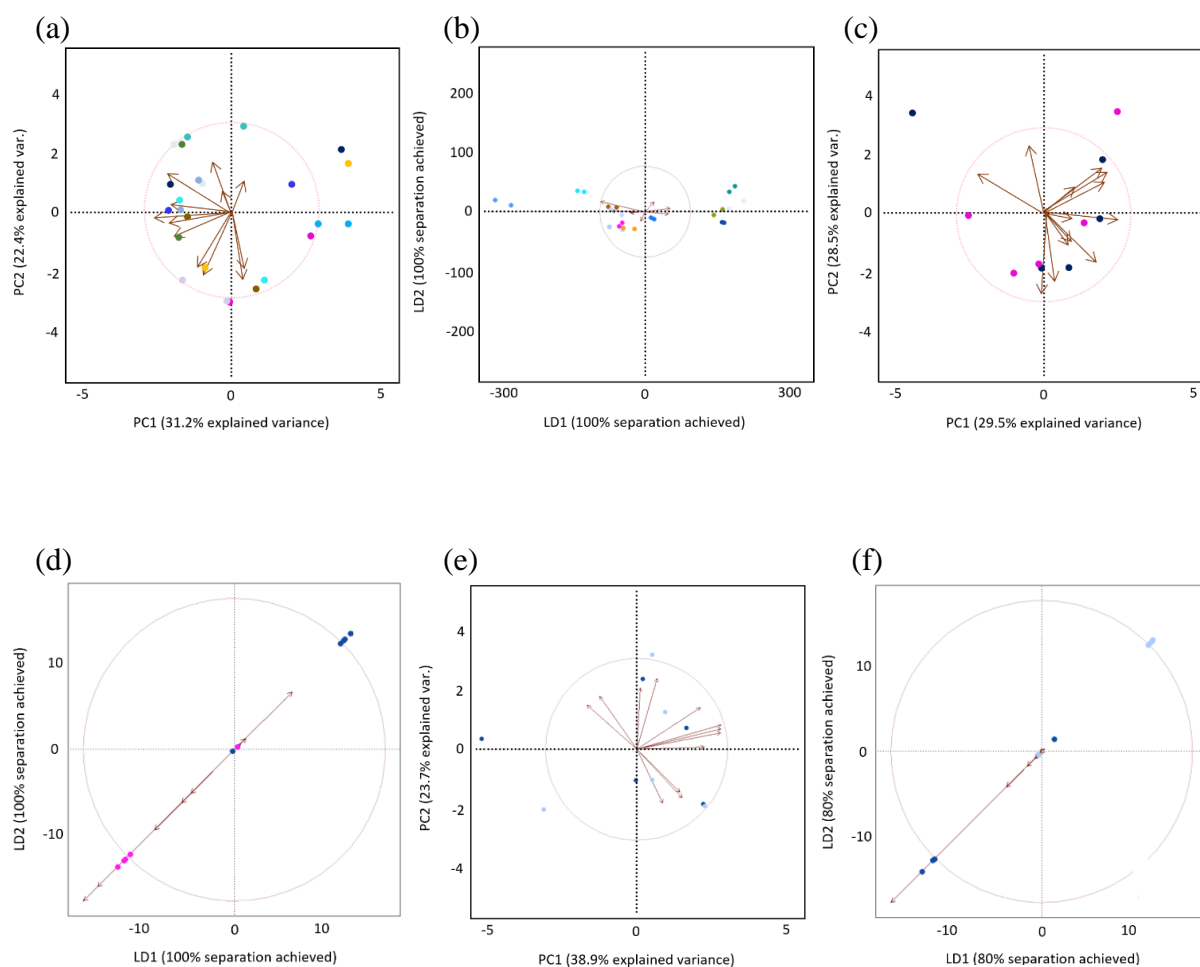


Figure 1. (a, c, e) Principal components analyses plots demonstrating the PC1 and PC2 loadings for reduced dimensionality of Tasmanian devil anal scent gland component compounds. Each red arrow indicates one chemical compound identified by the pre-processing and 3-blank protocol method. (b, d, f) Discriminant function analyses plots emphasizing the LD1 and LD2 loadings giving separation to respective scent marks. Plots (a, b) show male individuality compound loadings. Each dot represents a different sample and there are 2 samples for each individual distinguished by color. Plots (c, d) depict the difference between male and females. Each dot represents a different individual and the 2 colors represent the 2 variables being compared (i.e. male = blue, female = pink). Plots (e, f) depict the difference between sample collected from males during the breeding season versus samples taken from males during the non-breeding season. Each dot represents a different individual and the 2 colors represent the 2 variables being compared (i.e. breeding = dark blue, non-breeding = light blue).

Methods – Part II

Study Population

This study was conducted on Tasmanian devils located in a captive-breeding center in Cressy, Tasmania run by the Save the Tasmanian Devil Program. Data was collected from April 2015 – April 2017. All subjects were adults (> 1 year old) housed in individual enclosures to ensure accurate sampling. The Captive Research Advisory Group (CRAGS) gave approval for use of all animals in the captive breeding program. Animal Ethics approval was acquired through the Department of Primary Industries, Parks, Water and Environment (DPIPWE) committee.

Behavioral Data

In order to determine whether Tasmanian devils were capable of habituating to a conspecific's feces and discriminating between the habituated conspecific and a novel conspecific we followed standard habituation-discrimination procedures described by Halpin (1974). Subjects were $n = 12$ devils (6 males; 6 females) housed in standard enclosures. Feces Donors were 12 separate conspecifics, 6 adult males and 6 adult non-estrous females. Each of the 12 subjects was tested twice, once with male feces and once with female feces. Each subject was presented with feces from the same conspecific 4 times (habituation trials), separated by 10-minute observational periods where the subject's behavior was recorded using an ethogram (See Appendix) by an observer standing outside the enclosure. The fifth trial consisted of the simultaneous presentation of feces from 1) the donor to which the subject had been previously habituated and 2) feces from a novel conspecific that matched

the sex of the habituated donor. All observations were also video-recorded to ensure that records could be confirmed. The total number of habituation trials was 96 (4 trials* 2 sex donors*12 subjects). The total number of discrimination trials was 24 (1 trial*2 sex donors*12 subjects).

To determine whether individuals could identify familiar conspecifics, we compared the investigation rates of devils towards familiar and unfamiliar conspecific feces. Feces was collected from 24 donors, 6 familiar males, 6 familiar females, 6 novel males and 6 novel females. Familiarity was defined by a conspecific housed within 1 enclosure of the subject and unfamiliarity was defined as a conspecific from a different facility. Each subject participated in two trials, one trial with male feces donors and one with female feces donors. A trial consisted of a simultaneous presentation of feces from 1) a familiar conspecific and 2) an unfamiliar conspecific separated by 1 meter. Behaviors exhibited by the subject were recorded using an ethogram (See Appendix) by an observer standing outside the enclosure. All observations were also video-recorded to ensure that records could be confirmed. These observations lasted for 10 minutes after the donor feces was presented. The total number of discrimination trials was 24 (2 trials*12 subjects).

Feces Collection

Donor feces were collected directly from individual enclosures by keepers during routine husbandry procedures. Staff wore latex gloves and stored individual scats in zip lock bags that were immediately put into a -18 degree C freezer. Individual samples were labelled 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, but feces age was included as a covariate in analyses (see below). The range in

days for the habituation and discrimination experiment was 6-106 days and for the familiarization experiment it was 1-72 days.

Statistical Analysis

Habituation Experiment: A negative binomial regression model was conducted in STATA14 to compare frequency of feces investigation in the original fecal presentation with the second through fourth feces presentations and to account for the effects of donor type. In addition, a multi-level mixed effects regression model in STATA14 was used to analyze the duration of scent investigation in the original fecal presentation with the second through fourth feces presentations. To account for the potential impact of donor scent age, we included it as a possible confounding variable in each of the models along with sex, trial. In order to determine whether individuals were able to discriminate between a habituated scent and a novel scent, we added scent as a possible predictor in the discrimination analysis.

Familiarization Experiment: A negative binomial regression model in STATA14 was used to compare the frequency of feces investigation between familiar and unfamiliar donors across all trials. A multi-level mixed effects regression model in STATA14 was used to analyze the duration of feces investigation data between familiar and unfamiliar donors. To account for the potential impact of donor scent age, we included it as a covariate in each of the models along with sex, and feces type (familiar vs unfamiliar).

Results – Part II

Habituation and Discrimination

The results demonstrate that Tasmanian devils habituated to a conspecific's feces over multiple presentations. The negative binomial regression demonstrated that Tasmanian devils significantly decreased the frequency of investigation of donor feces (sniffing, anal dragging, handling, and mouthing) in trials two through four as compared to trial one (Trial 2: $z = -1.79$, $N = 12$, $p = 0.07$, Trial 3: $z = -3.15$, $N = 12$, $p = 0.002$, Trial 4: $z = -3.60$, $N = 12$, $p <$

0.001). In addition, the multi-level mixed effects regression model showed that they spent significantly less time investigating the donor feces (sniffing, anal dragging, handling, and mouthing) in trials two through four as compared to trial one (Trial 2: $z = -2.60$, $N = 12$, $p = 0.009$, Trial 3: $z = -4.33$, $N = 12$, $p < 0.001$, Trial 4: $z = -4.43$, $N = 12$, $p < 0.001$; Figure 2). Feces age and donor sex did not significantly influence investigation rate, however feces age significantly decreased the investigation duration of the devils ($z = -3.13$, $N = 12$, $p = 0.002$).

The discrimination analyses showed that Tasmanian devils can discriminate between habituated and novel conspecifics through the chemical cues in their feces. The negative binomial regression showed that Tasmanian devils investigated at a higher frequency and exhibited significantly more anal drags in response to novel devil feces compared to feces that they were habituated to (investigation: $z = -3.63$, $N = 12$, $p < 0.001$; anal drags $z = -2.46$, $N = 12$, $p < 0.014$). The multi-level mixed effects regression model demonstrated that they also had longer investigation durations and allocated more time to anal dragging in response to novel devil feces than to feces in which they were habituated to (investigation: $z = -3.21$, $N = 12$, $p < 0.001$; anal drag duration $z = -2.95$, $N = 12$, $p < 0.003$; Figure 3).

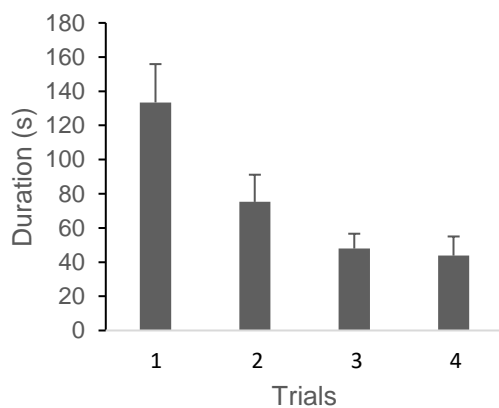


Figure 2. Average investigation durations between the 4 habituation trials across all study subjects. Error bars represent the mean duration \pm SD.

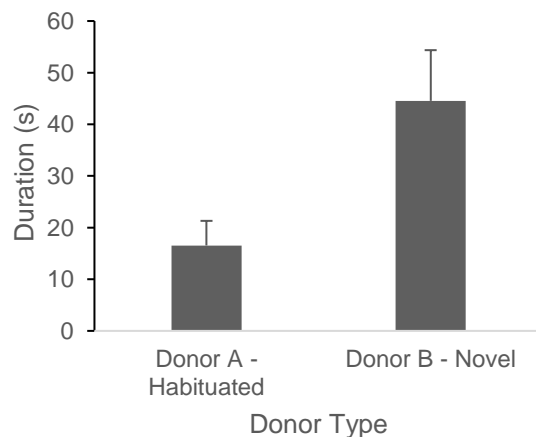


Figure 3. The average investigation duration between the habituated and novel scent in the fifth trial (presented simultaneously) across all study subjects. Error bars represent the mean duration \pm SD.

Familiar versus Unfamiliar

Results from the familiar versus unfamiliar experiment demonstrated that both familiarity and the sex of the donor influenced Tasmanian devil behavioral response to odor cues. The multi-level mixed effects regression model showed that there was a main effect of sex on time spent investigating the scent ($z = 3.74$, $N = 12$, $p < 0.001$; Figure 4). Specifically, both male and female Tasmanian devils spent significantly more time investigating female scent as compared to male scent. While there was no overall effect of familiarity on the time devils spent investigating feces ($z = -1.51$, $N = 12$, $p = 0.132$), there was a significant interaction between familiarity and donor sex on time spent investigating the feces ($z = -2.27$, $N = 12$, $p < 0.023$). The duration of anal dragging was significantly longer for unfamiliar conspecifics as compared to familiar conspecifics ($z = -2.39$, $N = 12$, $p = 0.017$, Figure 5), regardless of donor sex ($z = 0.76$, $N = 12$, $p = 0.449$). Scent age did not influence devil investigation behavior during the familiarization experiment ($z = 0.04$, $N = 12$, $p = 0.966$).

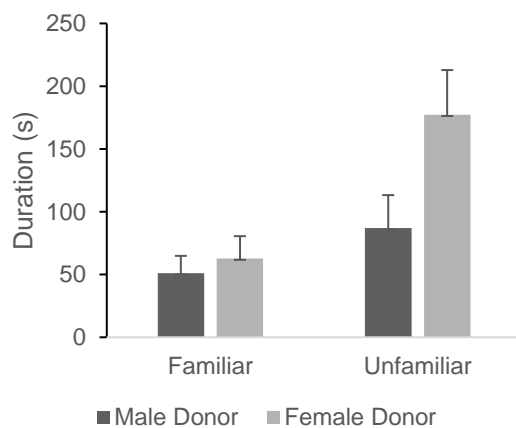


Figure 4. Average sniffing duration between familiar and unfamiliar donors across all study subjects. Error bars represent the mean duration \pm SD.

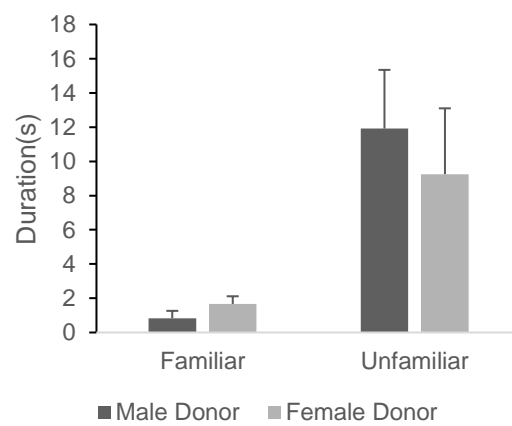


Figure 5. Average anal dragging duration between familiar and unfamiliar donors across all study subjects. Error bars represent the mean duration \pm SD.

Discussion

We found behavioral and chemical evidence that the anal scent gland secretion of the Tasmanian devil contains individual identity cues. This represents the first evidence of individual discrimination for a marsupial species. Tasmanian devil anal scent gland secretions are chemically complex substances composed of a large number of volatile compounds, and an individual's scent profile is defined by both the compounds that are present and the relative proportion each of these compounds contributed to the profile. Thus, if olfactory individual recognition in the Tasmanian devil is communicated by volatile compounds, it is most likely based on individually distinct composition of numerous compounds as has been suggested to be the case in other mammalian species (Hagey and MacDonald, 2003; Safi and Kerth, 2003; Smith et al., 2001) rather than simply the presence or absence of specific compounds. Apps (2013) provided clear evidence that mammalian semio-chemicals may code in ratios that delineate essential sender information. The classes of substances found in secretions from the anal scent gland of Tasmanian devils such as fatty acids, fatty acid esters, aldehydes and hydrocarbons (Buglass et al., 1990; Hofer et al., 2001) have also been found in secretions of other mammals (Burger, 2004) and some of these compounds are considered to be potentially relevant for the communication of individually specific information (Hagey and MacDonald, 2003; Yuan et al., 2004).

We also quantified significant variation in the response of individuals to fecal samples deposited by conspecifics suggesting that Tasmanian devils can distinguish between scent marks deposited by different individuals based on individual-specific characteristics. Tasmanian devils quickly habituated to conspecific feces and investigated novel feces more frequently and for longer, including sniffing, mouthing, handling and anal dragging. These results support the anecdotal evidence observed by management staff in captivity and suggests that scent cues play a critical role in their behavioral ecology.

The behavioral trials indicated that Tasmanian devils can become familiarized with conspecifics if presented with repeated presentations of olfactory cues and that devils of both sexes spent time investigating female feces more than male feces even outside of the breeding season. This suggests that feces is being utilized to assess female reproductive readiness. Female devils have been shown to have synchronous estrous initiated by daylight length and temperature (Keeley et al., 2017). These results, however, suggest that olfactory cues also play a role in synchronizing estrous cycles and may explain why females were interested in female feces more than male feces. The shorter latency and greater overmarking and scent-anointing response to fecal samples deposited by familiar individuals indicates that scent marking may be important for maintaining social networks within populations and may facilitate the transfer of information between group members. This is particularly relevant within the context of DFTD, as it is spread by bites and thus the level of direct physical aggression corresponds to the rate the disease spreads throughout a population. This evidence of distinction between familiar and unfamiliar individuals suggests that their interaction networks are more complex than once assumed and that olfactory communication may be a central component to reducing aggression interactions and therefore limiting the spread of DFTD.

If Tasmanian devils are able to discriminate between individually distinct cues within the chemical profiles of scent marks as our results suggest, then a communication mechanism would be required that allows for easy transmission amongst a population continually updates the recognition of identity odor cues of conspecifics, as these cues change with time. We suggest that latrines probably function as an important mechanism whereby animals can reappraise the scent profile of conspecifics, confirming their age, sex, breeding status and diet as well as their dispersal within a given area. These results support the idea that olfactory communication plays a key role in devil behavioral ecology and that latrine sites in the wild

are used as a communal source of information that persists in the environment. Scent cues at latrines likely communicate identity and sex of the scent donor and its possible that the age of the scent provides information about which devils continue to use the immediate area.

The use of individually distinct scent as a relatively persistent signal of an individual's presence in an area may provide selective advantages in the solitary, non-territorial society of the Tasmanian devil in which individuals directly interact irregularly. Tasmanian devils can travel long-distances making their typical home ranges 13.3 km² (Pemberton, 1990). Scent marks then, are ideal signals to relay continued presence within such a large area amongst a population that rarely interacts directly (Burgener et al., 2009). Additionally, scent mark degradation could also provide information on the time elapsed since the signal immersion and thus conspecifics could not only detect individuals present in an area, but also how recent that individual was physically present. Scent marking at information centers of the population such as the latrines is likely to be particularly useful in this context.

One additional consideration is the role these chemical cues have in Tasmanian devil mating behavior. As indicated by the behavior results that showed a heightened engagement with fecal samples from female donors in the familiarity trial. Females may be communicating critical information related to reproductive readiness or pregnancy that could facilitate mate selection in the wild. As Tasmanian devils are nocturnal and solitary, mating behavior is rarely observed. However, a species that relies so heavily on olfactory communication year-round is most likely communicating important information during the breeding season and utilizing the latrines to "advertise" such information across a population. This increases the evidence that Tasmanian devil latrine sites are acting as information centers relaying which individuals are in the area and who could be a potential mate. These findings greatly increase the understanding of the Tasmanian devil's rudimentary social

system and provide new avenues for applied management as efforts continue to restore wild populations.

Future Research

This research will provide insight into the social complexities of solitary, non-territorial top predators and the role scent plays in their communication systems. This study has shown both through chemical analysis and behavioral study that Tasmanian devils produce individually specific chemical cues and that their behavior suggests that they can detect these differences at the individual level. These results suggest a social system that is much more complex than previously documented and presents an opportunity for management strategies that specifically manipulate these olfactory cues to promote site fidelity and reduce dispersal post release. To gain a better understanding of how these cues can be manipulated it is critical to further investigate the impact of olfactory familiarization on aggression levels of conspecifics within a captive environment. This study could illuminate which individuals should be familiarized before release and how that familiarization should occur. It could also lead to management strategies that reduce the risk of DFTD transmission by reducing the rate of aggression interactions among resident conspecifics and releasees. In addition, such familiarization efforts could be applied to the captive breeding program to increase success of desired pairs, and thereby ensuring that genetic diversity is maintained within the captive population (Fisher et al., 2003; Swaisgood et al., 2000). All of these efforts have the potential to increase success of reintroductions efforts in the wild further aiding in the re-establishment of endangered Tasmanian devils in recently decimated areas.

Appendix

Ethogram for Feces Presentation Experiment

Defecate (DEF)	E	Passing of fecal material
Urinate (URI)	E	Eliminates urine
Sniff (SN)	S	Head lowered, nose near to the ground (about 5 cm or less), accompanied by sniffing and/or tasting. <ul style="list-style-type: none"> • Record stimulus sniff is directed towards (Familiar-novel feces)
Countermark (CM)	E	Defecate, urinate or anal drag on top of existing feces or urine; must contact/overlap bottom scent. Indicate clearly what is countermarked (e.g., which sample) and the type of scent used for countermarking.
Gape (GA)	E	Mouth open wide, showing teeth in a defensive posture. Indicate whether it is a full gape (GA - F) or half gape (GA - H)
Handle (HA)	E	The handling of the scat sample with forepaws
Mouthing (MO)	E	The placement of scat sample in mouth or licking of the sample
Anal Drag (AD)	S	Dragging rear on ground, depositing anal gland secretions <ul style="list-style-type: none"> • Record stimulus anal drag is directed towards (if within body length of sample) • Note distance dragged in devil body lengths (including tail) • Note anal secretions (white discharge)

E= Event: record frequency only (bout separation > 5 sec)

S= State: record start and stop times (duration)

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