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Differential Canid Predation of Translocated Juvenile Desert Tortoises (*Gopherus agassizii*) Using Chemical Signature Differences

Derek B. Hall[1,7], Bruce A. Kimball[2,3], Jennifer M. Germano[4,5], Eric M. Gese[6], and Jeanette A. Perry[1]

[1]Mission Support and Test Services, LLC, 2621 Losee Road, North Las Vegas, Nevada 89030, USA
[2]U.S. Department of Agriculture, National Wildlife Research Center, 3500 Market Street, Philadelphia, Pennsylvania 19104, USA
[3]Monell Chemical Senses Center, 3500 Market Street, Philadelphia, Pennsylvania 19104, USA
[4]Institute for Conservation Research, San Diego Zoo, 15600 San Pasqual Valley Road, Escondido, California 92027, USA
[5]Fauna Science Team, Department of Conservation, Munro Building, 186 Bridge Street Nelson, 7010, New Zealand
[6]U.S. Department of Agriculture, Wildlife Services, National Wildlife Research Center, Predator Research Facility, 4200 South 600 East, Millville, Utah 84326, USA
[7]Corresponding author, e-mail: halldb@nv.doe.gov

Abstract.—**Differential predation was observed in a population of 59 translocated juvenile Desert Tortoises (*Gopherus agassizii*) of known sex during a juvenile translocation survival study between September 2012 and November 2017. The main source of mortality was attributed to Coyote (*Canis latrans*) and Kit Fox (*Vulpes macrotis*) predation. Predation was skewed with higher female mortality than male mortality. We tested the hypothesis that juvenile females smell different than males, which leads to increased canid predation. We also explored differences in chemical signatures of resident adult female and male Desert Tortoises. We collected oral, cloacal, and chin/forelimb swabs from translocated juvenile and resident adult tortoises during fall 2015 and fall 2017 and analyzed them using headspace gas chromatography/mass spectrometry to determine potential differences in the chemical signatures of volatile compounds. Standardized chromatographic peak responses were subjected to Analyses of Variance (ANOVA). For development of artificial scents, mean responses were calculated for each juvenile tortoise from standardized responses representing all collections, and grand means were determined for males and females. Collections of volatiles differed significantly according to age and/or sex depending on the body location of collection. Among the plausibly endogenous volatiles that differed by age, many of them are alcohols. We conducted two field trials using captive Coyotes and one field trial partially within the translocation area to test if Coyotes showed a preference for female or bias against male synthesized scent. No consistent preference or bias was shown, suggesting that no innate preference for female odor was evident.**

Key Words.—mortality; predation ecology; sex bias; survival; translocation

Introduction

The Mojave population of the Desert Tortoise (*Gopherus agassizii*) north and west of the Colorado River in the USA is protected as a threatened species under the U.S. Endangered Species Act due to declining populations (U.S. Fish and Wildlife Service [USFWS] 1990). Predation is one of the main factors contributing to continued population declines (USFWS 2011; Berry and Murphy 2019). While predation by a wide variety of predators has been summarized on Desert Tortoises at different life stages (Berry and Murphy 2019), information on predation ecology (e.g., how do predators find Desert Tortoises, how do Desert Tortoises respond to or interact with predators) is lacking. A major source of mortality documented in several studies has been from canid predation, primarily Coyotes (*Canis latrans*; Peterson 1994; Esque et al. 2010; Lovich et al. 2014; Nagy et al. 2015), Kit Foxes (*Vulpes macrotis*; Kelly et al. 2019) or both (Nussear et al. 2012; Germano et al. 2017; Kelly et al. 2021). Luckenbach (1982) suggests that Coyotes are the major predator of adult Desert Tortoises. Similarly, the main source of mortality (77%; 24 of 31 mortalities) after 5 y of a long-term survival study of 59 translocated juvenile Desert Tortoises of known sex was attributed to Coyote and Kit Fox predation (Hall and Perry 2018). Surprisingly, results indicated a large difference in predation between sexes with nearly 2.5 times more females being depredated than males (17 versus seven). Germano et al. (2017) reported findings from the first year of this study and Hall and Perry (2018) summarized study findings related to survival after 5 y. Survival of translocated juveniles of unknown sex over a 3-y period (2005–2008) were evaluated by Nagy et al. (2015) with 32% survival documented. Most mortalities of smaller

Desert Tortoises (< 110 mm midline carapace length [MCL]) were attributed to Common Ravens (*Corvus corax*), and the main predator of larger juveniles (> 110 mm MCL) was Coyotes.

Differential predation in adult Desert Tortoises was recorded by Esque et al. (2010) who found that females were more likely than males to be killed by Coyotes. Higher female mortality from Cougar (*Puma concolor*) predation was reported by Riedle et al. (2010) who thought this may be attributed to female Desert Tortoises being active earlier in the season than males in the Sonoran Desert where their study took place. Reasons for higher canid predation on either adult or juvenile female Desert Tortoises have not been investigated before. Esque et al. (2010) mention that higher adult female predation by Coyotes was counter to what might be expected given that adult male Desert Tortoises have larger home ranges and generally move greater distances and they concluded that there were unlikely to be sex specific behaviors that would afford differential survival from Coyote predation.

Reptiles rely more on their chemical senses than any other vertebrate class, and behavioral studies and anecdotal observations suggest that chemical cues (sex pheromones) are important in the communication and reproduction of many reptiles (Martin and Lopez 2011). Other researchers have suggested that chemical cues from conspecifics play a role in influencing Desert Tortoise movement and burrow use patterns (Patterson 1971; Berry 1986; Bulova 1997). Terrestrial tortoises (Testudinidae) appear to have two primary sources of pheromones, which include the cloacal glands and the mental or chin glands (Mason 1992; Bulova 1997). The cloaca has been shown to be a source of conspecific chemical cues in many vertebrate species (references in Birch 1974) and male tortoises smell the cloacal area of females during courtship (Weaver 1970; Auffenberg 1977). Martin and Lopez (2011) note that the chemical composition of cloacal secretions and feces remains undescribed. In contrast, Rose et al. (1969) studied chin gland secretions of four species of *Gopherus* and found they contained phospholipids, triglycerides, free fatty acids, and cholesterol. They thought that although it is not known which of the gland components elicit an olfactory response, the fatty acids are likely involved and have characteristic odors that warrant further investigation. They also determined that chin glands are functional in females of all four species of *Gopherus*, and that glandular secretions of females contained a cathodal migrating protein not found in males.

An effort to further isolate and characterize the fatty acids from chin gland secretions of male Texas Tortoises (*Gopherus berlandieri*) found the presence of caprylic, capric, lauric, myristic, palmitic, palmitoleic, stearic, oleic, and linoleic fatty acids in the secretions (Rose 1970). He also studied male and female behavior of tortoises in response to a fatty acid solution painted on a plaster model of a tortoise and found the fatty acid composition served as an olfactory cue which elicited combat behavior (i.e., ramming) and not courtship in other males and a mating attraction from females. Based on these observations, the author concluded that there may be sexual differences in either the fatty acid composition or percentage composition on individual acids but all attempts to secure sufficient amounts of female secretions for fatty acid analyses failed due to the extremely small size of the female chin glands. Similarly, Alberts et al. (1994) were unable to collect secretion samples from females due to small glands.

A study investigating the social significance and chemistry of chin gland secretions in the Desert Tortoise concluded that both males and females discriminated between the chin gland secretions of familiar and unfamiliar male conspecifics and revealed the presence of 12–17 protein components ranging in size from 25,000 to 115,000 Daltons with slight individual differences in the number and size of high molecular weight components (Alberts et al. 1994). Studies using domestic detection Domestic Dogs (*Canis familiaris*) to find Desert Tortoises further emphasize that Desert Tortoise odor is a chemically un-described odor signature that should be studied more (Cablk et al. 2008; Mary Cablk and Russell Harmon, unpubl. report). In one study, detection Domestic Dogs detected Desert Tortoises of all sizes with no preference for female or male Desert Tortoises (Cablk et al. 2008). Cablk and Heaton (2006) found that wiping the tortoise neck and front legs with gauze was sufficient to capture enough scent to be able to train the dogs to identify a tortoise.

Given that tortoises use chemical signatures in their interactions with each other and the documented higher canid mortality on female juvenile Desert Tortoises, we suggest some possible explanations for this phenomenon. For example, it is possible that female juveniles could spend more time above ground, or travel farther, which makes them more susceptible to predation. Hall and Perry (2018) evaluated this hypothesis, however, and found that while male and female juvenile tortoises moved similar distances, females spent more time in their burrows. It is possible then that scent accumulation in burrows might lead to increased attraction of predators, and females spending more time in burrows also might attract predators. Only four of 24 predation events, however, documented dug up burrows, although all four burrows were those of females (Hall and Perry 2018). These were all burrows where the tortoises had spent the winter, which could have accumulated female tortoise scent. One might expect the same result from the 13 other females that were depredated, but this was not the case. Another possible explanation is that females smell differently to predators than males. This difference may attract or repel canid predators, or canids might be able

to associate an odor with female tortoises, which in some way confers an advantage to a predator by eating females rather than males.

Because canids use olfaction as one of their main senses to find prey (Wells and Lehner 1978), we determined that this concept was worth investigating. To better characterize chemical signatures in both male and female translocated juvenile and resident adult Desert Tortoises and to determine if they differed between sex and age, we collected oral, cloacal, and chin/forelimb samples and analyzed them for specific chemical signatures, specifically volatile compounds. During three field trials, we tested the hypothesis that chemical signatures differed between sexes of translocated juveniles. We predicted that female and male chemical signatures would be different, and we also predicted that canid predation would be higher on females than male tortoises.

Materials and Methods

Study area: sample collection and field trial 3.—The Nevada National Security Site (NNSS) is located in south-central Nevada, USA, approximately 105 km northwest of Las Vegas, and encompasses approximately 3,561 km^2 (Fig. 1). It is in an area of southern Nevada that lies between the Great Basin Desert and the Mojave Desert as defined by Jaeger (1957). The NNSS land has been withdrawn from public use since the 1950s as a U.S. Department of Energy Reservation, and a majority of the site (90%) has remained undisturbed. Our study area encompassed the southern one-third of the NNSS, which coincides with the known Desert Tortoise habitat on the site (Fig. 1). Relative Desert Tortoise abundance is low (3.9–17.4 tortoises per km^2) based on multiple surveys over several decades (EG&G/Energy Measurements 1991; Mueller and Zander 1994; Woodward, R., K.R. Rautenstrauch, D.B. Hall, and W.K. Ostler. 1998. The Relative Abundance of Desert Tortoises on the Nevada Test Site within Ecological Landform Units. EGG 11265-2039 UC-702, Available from https://www.osti.gov/servlets/purl/10121823; USFWS 2019). Within the study area, we selected three sites in the western portion of Area 22 for the release of juvenile Desert Tortoises, which then dispersed up to 6 km away (Fig. 1). The resident adult Desert Tortoises were opportunistically captured at various locations in the study area during a separate but concurrent study (Fig. 1). We conducted Field Trial 3 along an obscure, two-track dirt road that was located partially through release Site 2 (Fig. 1). Dominant vegetation consists of Creosote Bush (*Larrea tridentata*) and White Bursage (*Ambrosia dumosa*) in the valleys, lower bajadas, and broad drainages with Blackbrush (*Coleogyne*

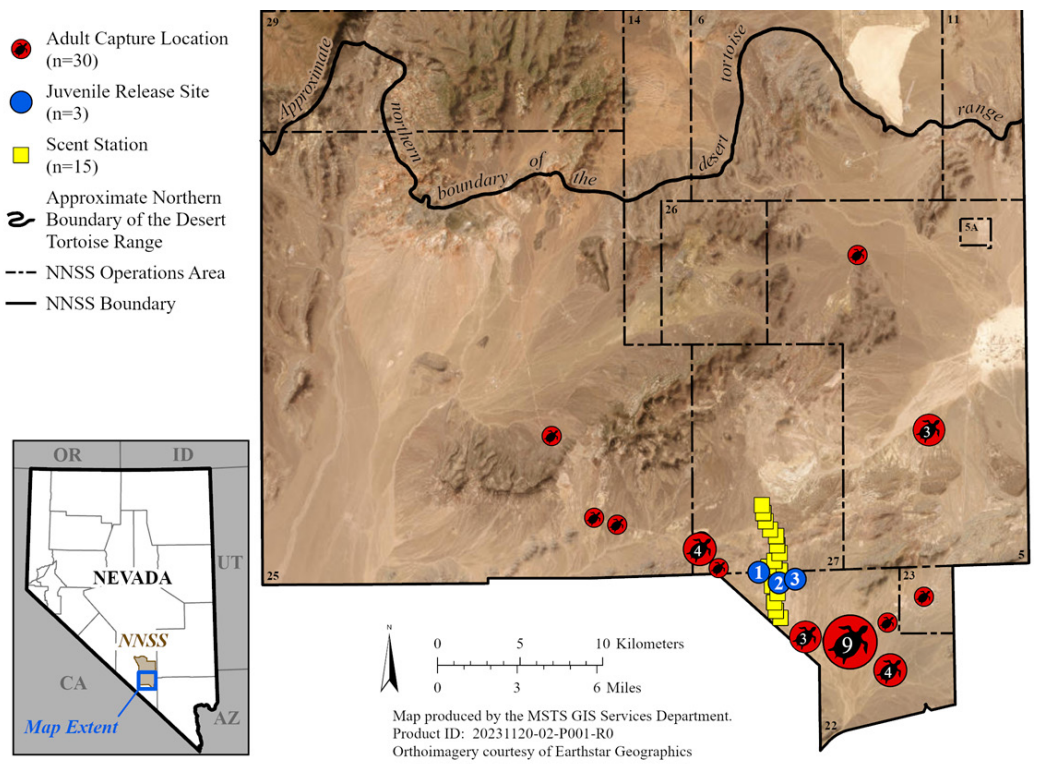

Figure 1. Study area including Desert Tortoise (*Gopherus agassizii*) habitat, release sites of translocated juveniles, capture locations of resident adults, and scent station locations on the Nevada National Security Site (NNSS), USA.

ramosissima) in the upper bajadas and upland areas. Elevation at the site ranges from 823 to 1,488 m. Average annual precipitation for the study area is about 12 cm (Soule 2006) and the climate is characterized by hot, dry summers and cool, dry winters with most of the precipitation coming during the winter and some during the summer monsoon season.

Study area: field trials 1 and 2.—We made behavioral assays with captive Coyotes at the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, National Wildlife Research Center, Millville Predator Research Facility, near Millville, Utah, USA. Coyotes were housed in 0.1 ha pens and provided a daily ration of 650 g of commercial Mink food (Fur Breeders Cooperative, Logan, Utah, USA) with water provided *ad libitum*.

Study animals.—On 21 September 2012, we translocated 59 juvenile Desert Tortoises of known sex (29 female, 30 male) estimated to be < 10 y old and ranging in size from 99–151 mm MCL from the Desert Tortoise Conservation Center (DTCC) in Las Vegas, Nevada, USA, to three release sites near the southern NNSS boundary as part of a long-term survival study. We randomly assigned juveniles to each release site (20 each to Sites 1 and 2, 19 to Site 3) with nearly equal numbers of males and females placed at each site. We determined sex before release by measuring plasma testosterone levels using the protocol from Rostal et al. (1994). The histories and origins of our animals were variable with some tortoises hatched at the DTCC, and others acquired through a hotline that accepted tortoises from the general public. We determined that tortoises were clinically healthy with no signs of nasal exudate for 90 d, negative results for *Mycoplasma agassizii* and *M. testudineum* antibodies by an ELISA assay, and tortoises were able to pass an official DTCC translocation screen (Bruce Rideout, unpubl. report) that assessed them for disease indicators, body condition scores, and other indices of health.

To the first costal scute of each tortoise, we affixed a very high frequency (VHF) radio transmitter (Model PD-2, 3.6 g, 6-mo battery life or RI-2B, 9.6 g, 12-mo battery life; Holohil Systems Ltd., Carp, Ontario, Canada) that were < 10% body mass of the tortoise. We tracked tortoises using radio telemetry at least weekly during March to October and monthly during November to February using a three-element Yagi antenna and receiver (Model R1000; Communications Specialists, Inc., Orange, California, USA). We changed transmitters each spring and/or fall through fall 2017 and we rotated placement of the transmitters between the left and right side of the carapace when we replaced a transmitter.

We opportunistically captured 30 resident adult Desert Tortoises ranging in size from 180–306 mm MCL between 10 May 2012 and 7 October 2015. We affixed to the carapace of each adult tortoise a VHF transmitter (Model RI-2B, 24-mo; Holohil Systems Ltd.) and a Global Positioning System (GPS) logger (Model G30L; Advanced Telemetry Systems, Inc., Isanti, Minnesota, USA, or a Model GT-120 i-GotU USB GPS Travel and Sports Logger; Mobile Action, New Taipei City, Taiwan). We tracked adults similarly to the juveniles. All juvenile and adult Desert Tortoises were handled according to USFWS guidelines (USFWS 2009b) by USFWS approved Desert Tortoise biologists.

We used 10 Coyotes in 2018 (five females, five males) and 12 Coyotes in 2019 (six females, six males) to assess preference or avoidance of synthesized juvenile female and male tortoise scent. These animals ranged in age from 2–7 y old. All animals were of reproductive age, and had similar history of vaccinations, feeding, and animal care.

Sample collection.—For cohort 1, we collected oral, cloacal, and chin/forelimb swabs to analyze the chemical composition of volatile compounds from 27 juvenile Desert Tortoises (19 males, eight females) between 24 September and 14 October 2015 and 27 adult Desert Tortoises (10 females, 16 males, one unknown sex) between 23 September and 21 October 2015. For cohort 2, we took additional samples from 26 juveniles (18 males, eight females) between 18 September and 10 October 2017 and 12 adults (nine males, two females, one unknown sex) between 6 September and 3 October 2017. We collected oral samples of saliva with a sterile, cotton-tip applicator on a wooden stick. The applicator was gently swabbed multiple times around the inside of the mouth of the tortoise. We collected cloacal samples of cloacal contents with the same type of cotton-tip applicator, which was inserted gently into the cloacal opening and swabbed multiple times around the inside of the cloaca. We took chin/forelimb samples by rubbing a circular cotton patch (about 50 mm diameter) under the chin where the chin glands are located and then on the front of the forelimbs. We placed each sample into a 10-mL vacutainer, sealed samples with a rubber stopper, placed them in a freezer until shipped, and shipped the samples frozen with ice packs overnight to the Monell Chemical Senses Center in Philadelphia, Pennsylvania, USA, for analysis. Sterile cotton-tip applicators, empty 10-mL vacutainers with rubber stoppers, and new round cotton patches were also provided for quality control.

Samples from the two cohorts were analyzed separately using headspace gas chromatography/mass spectrometry to determine if any chemical differences were detected. Cotton patches and applicator swabs were individually placed in 20-mL sample vials with septa crimp-seals and subjected to dynamic headspace analysis using a HT3 dynamic headspace analyzer (Teledyne Tekmar, Mason, Ohio, USA) outfitted with Supelco Trap K Vocarb 3000 trap (Sigma-Aldrich Co., St. Louis, Missouri, USA).

The sample vial was maintained at 40° C, swept with helium for 10 min (flow rate of 75 mL/min), and the volatiles collected on the thermal desorption trap. Trap contents were desorbed at 265° C directly into a Thermo Scientific ISQ single-quadrapole gas chromatograph-mass spectrometer (Thermo Scientific, Waltham, Massachusetts, USA) equipped with a 30 m × 0.25 mm id Stabiliwax®-DA fused-silica capillary column (Restek, Bellefonte, Pennsylvania, USA). The GC oven program had an initial temperature of 40° C (held for 3 min) followed by a ramp of 7.0° C/min to a final temperature of 230° C (held for 6 min). The mass spectrometer was used in scan mode from 33 to 400 m/z.

Baseline correction, noise elimination, and peak alignment of the chromatographic data were achieved using MetalignTM (Lommen 2009). Resulting multivariate data (consisting of all mass spectrometric responses exceeding a defined threshold at each scan event) were further processed with the MSClust tool for mass spectra extraction and generation of individual selected ion chromatogram peak responses (Tikunov et al. 2012). The resultant dataset consisted of a single response for all peaks identified in the chromatograms and was suitable for statistical analyses. All peaks were tentatively identified by their spectra in comparison to the National Institute of Standards and Technology standard mass spectral database.

Chemical analysis.—We identified 63 chromatographic peaks among the samples of cohort 1 that could be attributed to the biological collections by comparison to chromatograms of quality control blank patches and applicators. Similarly, 61 peaks were uncovered during analyses of cohort 2 samples. We identified 33 compounds common to both cohorts, and examination of age and sex differences as well as artificial scent development focused on these 33 compounds. We standardized peak responses by dividing each individual peak response by the total of 33 peak responses in each sample. Data were normal with equal variances, and we analyzed standardized peak responses using Analyses of Variance (ANOVA) with the MIXED procedure in SAS (SAS Institute Inc., Cary, North Carolina, USA). Age (adult or juvenile) and sex (male or female) were fixed effects. We analyzed responses from the three body locations (oral, cloacal, chin/forelimb) separately. To account for conducting 33 univariate tests of individual volatiles, we used the false discover rate controlling procedure (Benjamini and Hochberg 1995). For development of artificial scents, we calculated mean responses for each juvenile Desert Tortoise from standardized responses representing all collections (i.e., oral, cloacal, and chin/forelimb swabs) and we determined grand means for males and females.

Field trials 1 and 2.—To test if differences in chemical signature influenced differential canid predation on female and male juvenile Desert Tortoises, we conducted a field trial (Field Trial 1) 27–29 September 2018, at the Millville Predator Research Facility. We infused synthesized juvenile female and male Desert Tortoise scent diluted with ethanol (Table 1) into standard scent tabs (Pocatello Supply Depot,

Table 1. List of 33 chemicals common to both sets of collection cohorts including 19 used to synthesize female and male juvenile Desert Tortoise (*Gopherus agassizii*) scent and their respective concentrations (mL) used in canid bioassay trials and 14 chemicals considered exogenous in nature, unavailable commercially, or unknown which were not used in the synthesis of tortoise scent. These are at the bottom of the table and marked with an asterisk (*). Ethanol was used to dilute the tortoise scent concentration in Field Trial 1 and as the control stimulus in Field Trials 1 and 2.

Compound	Female Odor (mL)	Male Odor (mL)	Control (mL)
Acetic acid	4.000	3.700	--
Acetophenone	0.019	0.014	--
Benzaldehyde	0.047	0.047	--
Butanol	0.148	0.125	--
2-n-Butyl furan	0.020	0.027	--
p-Cymene	0.009	0.014	--
Decanal	0.179	0.118	--
2-Decenal	0.013	0.010	--
Ethanol	18.40	18.90	25.00
3,5-Heptadien-2-one, 6-methyl	0.007	0.006	--
2-Heptenal	0.035	0.039	--
5-Hepten-2-one, 6-methyl	0.028	0.022	--
Hexanal	0.385	0.408	--
Hexanol	0.203	0.173	--
Nonanal	0.170	0.147	--
2-Octenal	0.027	0.030	--
Octanal	0.114	0.081	--
Pentanol	0.788	0.814	--
2-Pentyl furan	0.070	0.075	--
Phenol	0.330	0.226	--
Caprolactone*	--	--	--
2-Chloroethanol*	--	--	--
Dodecane*	--	--	--
Ethyl benzene*	--	--	--
2-Methyl-1-pentanol*	--	--	--
2-Methyl-2-propanol*	--	--	--
3-Methyl-2-butenal*	--	--	--
Naphthalene*	--	--	--
1-Octanol*	--	--	--
o-Xylene*	--	--	--
1-Penten-3-ol*	--	--	--
Styrene*	--	--	--
Toluene*	--	--	--
Unknown*	--	--	--

Pocatello, Idaho, USA) made of plaster of Paris. We also presented a control tab diluted with ethanol that had no Desert Tortoise scent added. We presented the tabs to 10 captive Coyotes (five females, five males) in a choice trial to determine if they showed any preference. We randomly assigned one male scent tab, one female scent tab, and one control tab to a location about 20 m apart inside a clover pen (0.1 ha in size) containing a single Coyote. The scent tab was set on the ground within 0.5 m of the fence. We left tabs in place for about 24 h. We secured motion-activated cameras (Trophy Cam HD Trail Camera; Bushnell, Overland Park, Kansas, USA) to the fence approximately 3 m off the ground and oriented so the scent tab was within the field of view of the camera. We set the cameras to record a 20-sec video clip each time the camera was triggered and a minimum 1-min time lapse between video recordings. We viewed video clips, and we tallied the number of visits, number of investigations, and duration of investigations to the nearest second for each Desert Tortoise scent (female, male) and control for each Coyote. A visit was defined as each time a Coyote entered the field of view and an investigation was when a Coyote directed its attention to the scent tab (e.g., sniffing, scent marking). We conducted Field Trial 2 16–19 September 2019 at the Millville Predator Research Facility using the same methods as Field Trial 1, except we did not dilute the synthesized juvenile Desert Tortoise scent with ethanol before it was infused into the scent tabs, and we used 12 captive Coyotes (six females, six males).

Field trial 3.—The captive Coyotes at the Millville Predator Research Facility we used in field trials 1 and 2 were naïve to Desert Tortoises; therefore, we conducted a third field trial (Field Trial 3) with wild canids at the NNSS in Desert Tortoise habitat under the assumption that Coyotes and Kit Foxes in this area had encountered Desert Tortoises or their scent. Using a protocol used to census Coyotes adapted from Linhart and Knowlton (1975) and Roughton and Sweeny (1979, 1982), we conducted this trial from 30 October to 7 November 2019. We used the same formulation of female and male Desert Tortoise scent tabs that were used in Field Trial 2 in this trial. We set up paired stations with female and male scent tabs randomly placed on opposite sides of a dirt road at 15 locations, spaced about 500 m apart. We cleared a 1-m^2 area to make animal tracks more visible in the dirt, and we placed the scent tab in the middle of this cleared area. We checked sites daily for 9 d, except for one 2-d check over the weekend. During each check, we inspected cleared areas for canid tracks and then cleared all tracks. We identified tracks to species using illustrations in Murie (1975).

Data analysis of field trials.—We analyzed video clips and we recorded and summed the number of visits, number of investigations, and duration of investigations for each scent choice-Coyote combination for field trials 1 and 2. We calculated relative frequency by dividing the raw number for each scent choice by the total number for each Coyote. We used Goodness-of-fit and Chi-square analyses to test for differences among the female scent, male scent, and to control for the number of visits and investigations. Data were normal with equal variances, and we used ANOVA to test for differences among the female scent, male scent, and control for duration of investigations. Statistical significance was set at $\alpha = 0.05$. Due to low numbers of subjects for field trial 3, the results are limited to summaries rather than statistical analysis. This includes the number of visits to each scent choice and total number of canid tracks within the 1-m^2 area by species.

Results

Chemical analysis.—Of the 33 identified compounds shared between samples from both cohorts (Cohort 1, samples collected fall 2015; Cohort 2, samples collected fall 2017), 14 were considered exogenous in nature, were unavailable commercially, or unknown (Table 1). Recipes employing the remaining 19 compounds listed in Table 1 were determined for female and male scents through exploration of peak responses produced from sources of each compound analyzed individually. The volatiles collected from the chin/forelimb location differed significantly by age ($F_{32,2739} = 2.89$, $P < 0.001$), but not by sex ($F_{32,2739} = 0.51$, $P = 0.989$) or the interaction of age and sex ($F_{32,2739} = 1.12$, $P = 0.288$). The standardized responses of several individual volatiles (Table 2) were significantly different between adults and juveniles while accounting for multiple comparisons (i.e., using the false discover rate controlling procedure). Like the chin/forelimb volatiles, oral volatiles differed significantly by age ($F_{32,2706} = 1.76$, $P = 0.005$), but not by sex ($F_{32,2706} = 0.39$, $P = 0.908$) or their interaction ($F_{32,2706} = 0.51$, $P = 0.990$). No individual oral volatiles were significant when accounting for multiple comparisons. Cloacal volatiles differed significantly with both age ($F_{32,2739} = 2.28$, $P < 0.001$) and sex ($F_{32,2739} = 1.70$, $P = 0.008$), but not the interaction ($F_{32,2739} = 0.50$, $P = 0.992$). Only one individual cloacal volatile, styrene, demonstrated a significant age effect when accounting for multiple comparisons (Table 2).

Field trials.—In Field Trial 1, Coyotes visited female scent and the control significantly more often than male scent ($\chi^2 = 17.08$, df = 2, $P < 0.001$; Appendix Table 1). No significant differences were detected among choices in the relative frequency of investigations ($\chi^2 = 0.978$, df = 2, $P = 0.613$) or the duration of investigations ($F_{2,27} = 0.26$, $P = 0.770$). In Field Trial 2, Coyotes visited female scent significantly more often than male scent or

Table 2. Individual Desert Tortoise (*Gopherus agassizii*) volatiles that differ by age from collections from different body locations.

Compound	Description	Chin/Forelimb	Cloaca
1-Penten-3-ol	Alcohol	Juvenile > Adult ($F_{1,83}$ = 9.90, P = 0.002)	
2-Methyl-2-propanol	Alcohol	Juvenile > Adult ($F_{1,83}$ = 11.42, P = 0.001)	
1-Pentanol	Alcohol	Adult > Juvenile ($F_{1,83}$ = 8.40, P = 0.005)	
Styrene	Exogenous		Adult > Juvenile ($F_{1,83}$ = 18.02, P < 0.001)
1-Hexanol	Alcohol	Adult > Juvenile ($F_{1,83}$ = 23.26, P < 0.001)	
2-Chloroethanol	Exogenous	Adult > Juvenile ($F_{1,83}$ = 14.45, P < 0.001)	
2-Octenal	Aldehyde	Adult > Juvenile ($F_{1,83}$ = 7.34, P = 0.008)	

the control (χ^2 = 5.99, df = 2, P = 0.050; Appendix Table 2). No significant differences were detected among choices in the relative frequency of investigations (χ^2 = 0.218, df = 2, P = 0.896) or duration of investigations ($F_{2,33}$ = 1.60, P = 0.217). In Field Trial 3, there were two Kit Fox visits to female Desert Tortoise scent, both at Station 2 on days 1 and 2 of the trial, and two visits to male Desert Tortoise scent, both at Station 5 on days 3 and 5 of the trial. We did not check Stations on day 4 so it remains unknown if the Kit Fox visit was on day 4 or day 5. We found 24 Kit Fox tracks at the female scent station and nine at the male Desert Tortoise scent station. We did not detect any Coyote tracks.

Discussion

Chemical analyses.—Prey seeking involves multiple sensory cues. Predators may detect the prey item from great distances via olfactory cues and investigate it. Investigative and consummatory behaviors may incorporate multiple sensory inputs (e.g., taste, odor, visual). In general, this is performed at a very short distance from the food item. The analytical tools employed in this study identified only those highly volatile chemicals that are detectable by olfaction and not phospholipids, triglycerides, cholesterol, or protein components found in other studies.

Using Headspace Analyses, many highly volatile compounds were observed in the samples collected from juvenile and adult Desert Tortoises. This was a complex suite of volatiles that differed by sex, age, and body location. These odorants, singly or in some combination, may serve as cues to foraging predators, especially for canids that have excellent odor memory capabilities in comparison to other mammals (Lo et al. 2020). Because we were interested in differential predation between female and male juvenile Desert Tortoises, we used chromatographic data to prepare synthetic scents of juvenile male and female Desert Tortoises for bioassays with captive and free-ranging mammalian predators. We did not test the predator response to synthesized scent from adult Desert Tortoises.

Our analyses clearly demonstrated that the collections of volatiles differed according to age and/or sex depending on the location of collection. The lack of significant individual volatiles suggests that the volatile effect of these chemicals is complex. That is, there are distinct patterns of volatiles that correspond to age or sex, but these patterns are not well-described by examination of individual volatiles. Among the plausibly endogenous volatiles that differed by age, many of them are alcohols. Many alcohols are products of lipid and fatty acid metabolism (Wishart et al. 2018) but may also be produced by the microbiome (Rojo et al. 2017).

Field trials.—Overall, the captive Coyotes showed little to no preference for female Desert Tortoise scent, male Desert Tortoise scent, or the control scent tabs. While more visits were made to the female scent tab during Field Trial 2, visits to the control tabs were about equal to visits to the female scent tab during Field Trial 1, suggesting there may be a slight preference for the female scent or weak bias against male scent. This is based on the number of visits, however, which was when a Coyote passed through the field of view of a camera. Presentation of the scent tabs was uniform and should not have influenced the number of visits. It is unknown if other factors were present that might have influenced the movement of Coyotes within the pen and thus influenced the number of visits to the different scent tabs. If a true preference for female Desert Tortoise scent or bias against male Desert Tortoise scent exists, this pattern should be exhibited even more strongly in the number of investigations (actual interaction with the scent tab) or duration of investigations. This was not the case because there were no significant differences in number of investigations or duration of investigations to female versus male versus control scent tabs in either Field Trial 1 or 2.

We suspect that the captive Coyotes may have simply been responding to novel items (i.e., scent tabs) placed in their environment rather than showing a real preference or bias for different Desert Tortoise scent. Across all scent choices, male Coyotes tended to investigate more and for longer periods of time than female Coyotes, which suggests male Coyotes may be more curious and react more strongly to novel objects

than female Coyotes. Heffernan et al. (2007) found male Coyotes investigated a large novel object (traffic cone) at a higher rate than female Coyotes, but time investigating the object was similar between sexes. Harris and Knowlton (2001) found males spent a greater amount of time within 5 m of a novel object and made more approaches towards the novel object than female Coyotes.

In Field Trial 3, canids did not show a preference for female or male Desert Tortoise scent with equal visitation by Kit Foxes to both scents. We found more individual Kit Fox tracks at the female scent, which may mean it spent more time investigating the area than at the male scent. We found low canid visitation at all stations, and we did not find sign from other Desert Tortoise predators (e.g., Bobcat, *Lynx rufus*, or American Badger, *Taxidea taxus*). This is likely due to low predator densities in the area. Camera trap data from the study area between 2017 and 2022 detected a coyote every 135 d and a kit fox every 125 d (unpubl. data). Based on results from a standard canid monitoring protocol (Linhart and Knowlton 1975; Roughton and Sweeny 1982), scent tabs are detectable by canids for many days (Webster and Beasley 2019), so we did not change the scent tabs over the 9-d sampling period.

We collected our samples during September and October, which coincides with the latter part of the mating season of Desert Tortoises (Berry and Murphy 2019). Chin glands were swollen on several of the adult male Desert Tortoises during collection but not on the adult females or juveniles of either sex. Based on behavioral studies, males are more aggressive especially during mating season (Rose 1970, Weaver 1970), and it is plausible but as of now undocumented that male tortoises exhibit more aggression than females during a predator attack, which could deter the predator. Clearly, more work is needed to understand both juvenile and adult Desert Tortoise response to predator attacks.

Summary.—Hall and Perry (2018) documented biased female mortality from canid predation on translocated juvenile Desert Tortoises. If higher juvenile female mortality is occurring in natural populations, this could lead to a decline in Desert Tortoise populations given the importance of females surviving to adulthood and recruiting into the reproducing population. Juveniles in natural populations should be studied to determine if differential predation is occurring and to document sex ratios for comparison with our results. Results from our study highlight the importance of documenting the sex of Desert Tortoises, notably juveniles, during translocation studies, as well as studies in natural populations. We recommend that in future juvenile translocations, perhaps up to twice the number of females should be released to account for potential increased mortality of translocated juvenile females. Collections of volatiles differed significantly according to age and/or sex depending on the body location of collection documenting different chemical signatures among adult and juvenile female and male Desert Tortoises. Data from the synthesized chemical scents and observations from all three trials suggest that although there are chemical differences between female and male juvenile Desert Tortoises, this does not account for increased predator attraction or curiosity toward female Desert Tortoises and therefore, would not account for increased predation of female Desert Tortoises observed in our study. Further research on canid predation ecology of Desert Tortoises is warranted.

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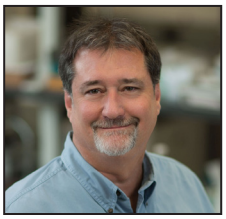
Derek B. Hall is a Wildlife Biologist with Mission Support and Test Services, Las Vegas, Nevada, USA, a contractor to the U.S. Department of Energy. He graduated with B.S. and M.S. degrees in Wildlife and Range Resources from Brigham Young University, Provo, Utah, USA, and has worked at the Nevada National Security Site, formerly the Nevada Test Site, for over 29 y. Derek has conducted studies on several sensitive species including the Western Burrowing Owl (*Athene cunicularia hypugaea*), Western Red-tailed Skink (*Plestiodon gilberti rubricaudatus*), and several bat species. His current research activities are focused on studying the abundance, distribution, and movement patterns of Cougars (*Puma concolor*) and native ungulates, studying survival and habitat use of a translocated population of juvenile Desert Tortoises, and revegetating disturbed Mojave Desert/southern Great Basin Desert ecosystems. (Photographed by Alexandria A. Wilson).



Bruce A. Kimball received his M.S. (Chemistry) from Arizona State University, Tempe, Arizona, USA, and Ph.D. (Ecology) from Colorado State University, Fort Collins, Colorado, USA. He conducts his research at the Monell Chemical Senses Center in Philadelphia, Pennsylvania, USA. The Kimball lab employs analytical chemistry, physiology, ecology, animal behavior, and statistics to improve the health and well-being of all species. Using trained biosensor animals and sophisticated analytical techniques to uncover chemical signals of disease, this research will bring diagnostic capabilities to under-served communities and potentially identify infectious diseases in animals before they crossover to humans and become pandemic. (Photograph courtesy of Monell Chemical Senses Center).

Jennifer M. Germano is a Wildlife Biologist in the New Zealand Department of Conservation Fauna Science Team, Nelson. She has a M.Sc. and Ph.D. from the University of Otago, New Zealand. Jennifer previously worked for the Desert Tortoise Conservation Center of the San Diego Zoo, California, USA. Her primary research interests are in the conservation management of amphibians and reptiles with a particular focus on wildlife translocations, mitigation measures for development, climate change, and Leiopelmatid frogs. Jennifer leads the Translocation Working Group of the Amphibian Specialist Group of the International Union for Conservation of Nature. She also works with and has a strong interest in empowering indigenous communities to work in conservation. (Photographed by Laken Gieger).

Eric M. Gese received a B.S. in Biology from the University of Texas - San Antonio, USA, and a M.S. and Ph.D. in Wildlife Ecology from the University of Wisconsin – Madison, USA. He is a Research Wildlife Biologist with the National Wildlife Research Center of the U.S. Department of Agriculture and is a Professor in the Department of Wildland Resources at Utah State University, Logan, USA. Eric has conducted research on carnivore ecology, behavior, and management for 40 y. (Photographed by Wayne Wurtsbaugh).

Jeanette A. Perry is a Wildlife Biologist with Mission Support and Test Services, Las Vegas, Nevada, USA, a contractor to the U.S. Department of Energy. She holds a B.S. in Ecology and Evolutionary Biology from the University of Nevada Las Vegas, USA, and is currently earning her M.S. from Colorado State University, Ft. Collins, USA, in their Fish, Wildlife, and Conservation Biology program. Her career has focused on recovery of the Mojave Desert Tortoise in southern Nevada. Her current interests include road ecology, sensitive/rare plants of Nevada, and conservation biology. (Photographed by Jennifer M. Germano).

Appendices

Appendix Table 1. Results from Field Trial 1 including number and relative frequency (in parentheses) of visits, investigations, and average duration of investigations (seconds) by scent choice-Coyote (*Canis latrans*) combination, 27–29 September 2018.

		Visits			Investigations			Duration (seconds)		
Pen No.	Coyote	Female	Male	Control	Female	Male	Control	Female	Male	Control
NI1	M1413	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0	0	0
SI2	F1060	7 (0.280)	7 (0.280)	11 (0.440)	3(0.25)	4 (0.333)	5 (0.417)	13	13	10
NI2	F1422	4 (0.364)	0 (0.000)	7 (0.636)	3 (0.333)	0 (0.000)	6 (0.667)	14	0	6
SI1	M1383	10 (0.172)	11 (0.190)	37 (0.638)	4 (0.154)	8 (0.308)	14 (.538)	6	6	7
SI4	F1360	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0	0	0
SI3	F1372	47 (0.887)	0 (0.000)	6 (0.113)	12 (0.706)	0 (0.000)	5 (0.294)	6	0	9
NI4	F1450	2 (0.250)	5 (0.625)	1 (0.125)	2 (0.250)	5 (0.625)	1 (0.125)	6	12	11
NI5	M1423	19 (0.373)	12 (0.235)	20 (0.392)	13 (0.351)	11 (0.297)	13 (0.351)	15	13	12
NI6	M1331	11 (0.407)	8 (0.296)	8 (0.296)	6 (0.429)	6 (0.429)	2 (0.143)	7	12	4
SI6	M1311	1 (0.038)	12 (0.462)	13 (0.500)	1 (0.071)	8 (0.571)	5 (0.357)	20	11	17
	Total	101 (0.390)	55 (0.212)	103 (0.398)	44 (0.321)	42 (0.307)	51 (0.372)	9	7	8

Appendix Table 2. Results from Field Trial 2 including number and relative frequency (in parentheses) of visits, investigations, and average duration of investigations (seconds) by scent choice-Coyote (*Canis latrans*) combination, 16–19 September 2019.

		Visits			Investigations			Duration (seconds)		
Pen No.	Coyote	Female	Male	Control	Female	Male	Control	Female	Male	Control
NI2	M1221	1 (0.250)	2(0.500)	1 (0.250)	1 (0.250)	2 (0.500)	1 (0.250)	6	3	13
NI4	M1703	9 (0.563)	4 (0.250)	3 (0.188)	5 (0.556)	2 (0.222)	2 (0.222)	16	6	6
NI6	F1200	2 (0.333)	0 (0.000)	4 (0.667)	0 (0.000)	0 (0.000)	1 (1.000)	0	0	14
SI2	M1351	3 (0.333)	3 (0.333)	3 (0.333)	1 (0.143)	3 (0.429)	3 (0.429)	13	18	10
SI4	F1620	61 (0.670)	11 (0.121)	19 (0.209)	10 (0.455)	7 (0.318)	5 (0.227)	8	9	11
SI6	F1370	19 (0.455)	4 (0.182)	8 (0.364)	2 (0.400)	1 (0.200)	2 (0.400)	5	7	20
NI1	F1610	2 (1.000)	0 (0.000)	0 (0.000)	2 (1.000)	0 (0.000)	0 (0.000)	7	0	0
NI5	F1250	9 (0.375)	7 (0.292)	8 (0.333)	2 (0.167)	5 (0.417)	5 (0.417)	13	12	11
NI3	M1611	17 (0.500)	7 (0.206)	10 (0.294)	5 (0.250)	7 (0.350)	8 (0.400)	12	9	14
SI1	F1600	6 (0.102)	25 (0.424)	28 (0.475)	2 (0.200)	4 (0.400)	4 (0.400)	19	12	10
SI3	M1623	10 (0.435)	7 (0.304)	6 (0.261)	5 (0.417)	4 (0.333)	3 (0.250)	13	11	13
SI5	M1615	3 (0.064)	36 (0.766)	8 (0.170)	3 (0.200)	4 (0.267)	8 (0.533)	17	9	18
	Total	133 (0.395)	106 (0.315)	98 (0.291)	38 (0.319)	39 (0.328)	42 (0.353)	11	8	12