# Tools and Technology



# An In Situ Vertebrate Bioassay Helps Identify Potential Matrices for a Predator-Based Synthetic Management Tool

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ABSTRACT To assist management and conservation needs, researchers have called for active kairomones to be elucidated and synthesized directly from animal exudates. However, the existing literature does not provide guidance on how to initiate this complex process. To our knowledge, composite synthetic predator scents that incorporate multiple compounds to accurately mimic the natural signal have not been produced. One approach to improve the accuracy of synthetics is to identify and recombine all major infochemicals within a benign solvent. Therefore, we tested 2 natural, pre-existing matrices for their potential as vehicles for delivery of a predator scent, dingo (Canis lupus dingo) urine, which causes a startle reaction among western gray kangaroos (Macropus fuliginosus), and avoidance by European foxes (Vulpes vulpes). We compared 2 putative backbone matrices-aged (3-yr old) dingo urine from a previously active lot, and 10% methanol-to a distilled water control. We used a novel fence-crossing assay to observe kangaroo interactions with both solvents and a negative control. Our assay allowed us to control for high feeding motivation by testing compounds away from the food source. We determined that neither free-ranging kangaroos nor European red foxes were adversely affected by either treatment matrix. Foxes were, however, attracted to the aged dingo urine, and were often observed scent-rolling in the inactive substance. Our results suggest that dilute methanol could be a possible matrix for predator-scent applications for kangaroos, while aged scents may act as an attractant for nontarget species, particularly canids. © 2012 The Wildlife Society.

KEY WORDS animal behavior, animal welfare, conservation behavior, deterrents, dingo, kairomones, kangaroos, semio-chemicals.

For the past 30 years, researchers have been calling for the accurate reproduction and resuspension of predator scents as tools to assist animal conservation and management needs (Martin and Fagre 1988, Sullivan et al. 1988, Parsons et al. 2007, Cox et al. 2010). While we recognize that the efficacy of scent applications are context-dependent (e.g., varies within target species [Apfelbach et al. 2005], by whether the animals share a natural history [Parsons et al. 2007], by the diet of the predator [Cox et al. 2010], and by season tested [Hayes et al. 2005]), scent applications may serve as a means to enhance both the conservation and welfare of free-ranging animals and may enrich the olfactory environment under zoo-based conditions (Clark and King 2008). These effects may be beneficial in the short-term until habituation develops (Murray et al. 2006), or indefinitely-when sensitization results in fear conditioning (Parsons and Blumstein 2010a, Gotz and Janic 2011) or

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<sup>2</sup>Present address: State University New York—Empire State College, Hartsdale, NY 10538, USA. when animals become resensitized to a fear cue following a period of unavailability (Teilmann et al. 2006).

Within Australia, dingo (*Canis lupus dingo*) scents (urine and feces) generate startle, vigilance, and avoidance behaviors by potential prey. Western gray (*Macropus fuliginosus*) and red kangaroos (*M. rufous*; Parsons et al. 2007, Parsons and Blumstein 2010*a*), brush-tail possums (*Trichosuras vulpecula*; Parsons and Blumstein 2010*b*), and European red foxes (*Vulpes vulpes*; Ritchie and Johnson 2009, Wallach et al. 2009) all respond adversely to dingo scents.

In addition to herbivore repellent properties, natural predator scents may affect other nontarget wildlife, including threatened species and meso-predators. For instance, the presence of dingo scents has been positively correlated with the abundance of endangered mallee fowl (*Leipoa ocellata*; Johnson et al. 2007) and yellow-footed rock wallabies (*Petrogale xanthopus*; Wallach et al. 2009), which suggests that dingoes displace other potential predators and possible competitors. When dingoes scent mark near European red foxes (Wallach et al. 2010), feral pigs (*Sus scrofa*), and goats (*Capra hircus*), these species tend to avoid the scent-marked areas (Dickman et al. 2009). Thus, dingo scents, and other truly natural predator scents,

may be utilized to assist native wildlife conservation. However there may be unintended consequences for strategically deploying biologically significant scents. For instance, feral dogs may be lured into a community at the same time that herbivores are being repelled (Michell and Kelly 1992).

Researchers have suggested that biologically active constituents, kairomones, be synthesized, or reproduced from predator by-products in order to influence herbivore patch selection (Cox et al. 2010, Parsons and Blumstein 2010*a*). This is required, in part, because there are so few dingoes where kangaroos are widespread (Newsome et al. 2001) and because unwanted meso-predators, such as foxes, avoid marked dingo territories (Dickman et al. 2009). This approach is minimally invasive, may reduce the need for culling in some instances, and in so doing, partly address welfare concerns (Fraser 2010, Littin 2010).

A better understanding of the mechanism for deterrence (e.g., identifying the chemicals that elicit a response) is necessary in order to maximize our ability to mimic the natural cue. Glandular exudates, urine, and feces advertise many interacting chemicals. Composite "scent codes," including gender, age, endocrine status, level of hunger, and genotype (Wyatt 2003, Brennan 2009, Ferrero and Liberles 2009), may all be deciphered by eavesdropping prey (Stowe et al. 1995, Parsons and Blumstein 2010*a*, *b*). Therefore, it is unlikely that one, or a few, active chemicals can mimic the complex information in recently voided predator scents (Wyatt 2003), and we are unaware of any pre-existing synthetic scents that aim to mimic the complete natural compound.

Single active constituents in predator odors have been isolated, identified, and trialed as herbivore repellents (Isopentynyl methyl sulfide [Epple et al. 1995]; 3,3-dimethyl-1,2-dithiolane [Burwash et al. 1998]; 2-propylthietane and 3-propyl-1,2-dithiolane [Sullivan et al. 1988]); however, these fractioned scents are rarely as effective as the original predator scent (Epple et al. 1995).

The process of synthesis is complex. For this reason, synthetically derived chemicals are imperfect and may not elicit the same response by herbivores that the natural scent evokes. In the case of kangaroos, the newly created compound should evoke the natural startle response where the scent may influence whether, and how long, the target species browse a particular food patch (Parsons and Blumstein 2010a). To help make the duplicated scent more accurate, it will be necessary to add many active chemicals in their appropriate ratios prior to deployment. This requires biologically inert solvents as a mechanism for resuspension and delivery. This natural approach may be more likely to elicit long term benefits. For instance, a natural predator cue may promote area-avoidance, and may also serve to resensitize herbivores to predator scents that have previously been habituated to (Gotz and Janic 2011). An unnatural (less accurately mimicked) scent may evoke lesser responses. We caution the reader that a less natural scent may appear to be effective at deterring animals, but may only be altering the palatability around the food source, as opposed to promoting vigilance and potentially altering herbivore behavior.

Furthermore, despite repeated calls to create accurate reproductions of predator scents (Martin and Fagre 1988, Sullivan et al. 1988, Cox et al. 2010), an in situ bioassay has not yet been produced to test the effects of repeated stimuli on the behavior of free-ranging target species, or to compare closely related homologs (closely related synthetics that can be compared to one another for their effects on multiple species). Therefore, it is necessary to consider novel assays where shy animals can be exposed repeatedly to scent treatments without compromising their welfare. Importantly, the assay should quantify vigilance (startle and/or retreat from cue) if we are to contrast these effects with noxious-based deterrence. When creating an assay, we sought to overcome a common experimental flaw, "high feeding motivation," where the attractiveness of an unnaturally inviting food patch might partly mask the inherent deterrent effect (Kimball et al. 2009). It is desirable to know when a repellent works in low-feeding-motivation situations, because most repellents are context-dependent and potentially synergistic when approached with multimodal strategies.

Our 2 target solvent matrices were selected among many potential alternatives because they naturally co-occur with target and nontarget species and were not known to attract, or repel, target species. It is important that natural, benign solvents are chosen in order to exclude potential neophobic effects of a compound. We chose dilute methanol, a slightly polar chemical that occurs naturally in ripened fruits, and aged dingo urine (collected and trialed in 2007). Aged urine smells like recently voided dingo urine to our nose; however, this compound has degraded over time and has lost its ability to influence kangaroo behavior (M. H. Parsons, unpublished data). This finding is consistent with the literature in that an indicator, or time stamp, of void is likely present (Wyatt 2003, Müller-Schwarze 2006), and the differential volatility of the signal may be one reason that canines regularly replenish their territorial markings. Aged urine was therefore a particularly interesting solvent in that we wondered if we could "reactivate" the diminished startle response if the appropriate chemicals were to be added back to the aged (degraded) backbone. We were not interested, however, in resuspending active (nonaged) urine; thus, it was unnecessary to consider whether either matrix would dilute fresh urine.

Specifically, in order to identify an appropriate solvent matrix, we asked the following questions: whether dilute methanol would increase the number of vigilant (visual scanning beyond the animal's reach while ears are erect; Parsons et al. 2007), startle (fleeing from the scent; Parsons and Blumstein 2010*a*, *b*), or luring behaviors of kangaroos or foxes, and whether aged dingo urine from a previously active batch would increase the numbers of vigilant, startle, or luring behaviors of kangaroo or foxes.

# **STUDY AREA**

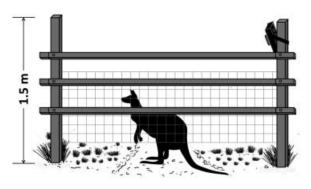
We conducted trials on the grounds of the Tibetan Buddhist Temple located in Herne Hill in the Swan Valley of Western Australia (31°49′26″S and 116°01′48″E) between 15 April and 4 May 2010. Between 30 and 50 free-living western gray kangaroos regularly crossed at 3 locations under a 1.5-m fence to browse ornamentals and native flora on the property. Prior to trials, >2,000 native tree seedlings, shrubs, and ornamentals were heavily grazed by kangaroos inside a 12.5-acre (5.06 ha) fenced area on the property.

# **METHODS**

There are no known in situ bioassays for free-ranging medium- or large-sized vertebrates. The current template for the repeated testing of medium or large vertebrates is based on captive pen trials (Kimball et al. 2009). Unfortunately, kangaroos may suffer a fatal capture myopathy when captured or placed in pens (Cox et al. 2010). Furthermore, tests with wild animals are widely considered a more accurate means to evaluate vigilance and repellency (Cox et al. 2010). This is especially important if the final application is meant to influence the behavior of wild animals.

Our fence-crossing bioassay was created to assess behaviors of free-ranging animals in their native home range, while minimizing repeated exposures to the same individuals. We selected a site with regular visitation by >30 target kangaroos. To minimize the effects of potential pseudo-replication on inferential statistical analysis (Hurlbert 1984), we chose to monitor a kangaroo crossing, via fixed camera (Stealth Prowler HD night-vision camera set to 60-s video mode), where individual kangaroos could be observed both arriving at, and leaving from, their preferred browse. We also chose to mount the camera and treatment away from the vicinity of preferred forage in order to eliminate "high feeding motivation" (Kimball et al. 2009) by limiting any interactions between the attraction of the food and potential attraction, or repellency, of the solvent.

A motion-sensing camera with high-definition video and audio (Fig. 1) was fixed where kangaroos daily cross under a fence to access preferred forage. The infrared detectors responded to motion as far as 30 m away. As a risk-aversion strategy, kangaroos favor the same crossing points and rarely deviate from a preferred path (Coulson et al. 2003). Kangaroos were never observed hopping over the fence on this property; kangaroos typically push under fences, rather



**Figure 1.** Fence-crossing bioassay in Herne Hill, Australia, May 2010. Fence is approximately 1.5 m high. Animals have dug under 3 areas of the fence to form crossing points. A 6-inch (15 cm) gap is necessary for a large male kangaroo to push under the fence. Kangaroos enter property to obtain preferred forage, and exit via same fence opening. Attractive forage is >50 m from the site. Camera (upper right) and burrowing trench (center) are visible.

than jumping a fence and risking injury (Coulson et al. 2003). The high-definition camera was mounted close enough to the path that differences in color and pattern of the pelage could be clearly observed in 60-second video clips of kangaroos (Jarman et al. 1989) and so that limb length, tail shape, and plumpness could be used to distinguish individual foxes.

During trials, a maximum of 17 kangaroos and 4 foxes were observed crossing under the fence line on a given night. We examined behavioral effects from 2 treatments, 10% methanol and degraded (3-yr old) dingo urine, along with distilled water-a negative control. A positive dingo urine control was not selected due to the difficulty and expense of collecting fresh urine, and due to the body of research where dingo scents have been shown to influence kangaroos (Parsons et al. 2007, Parsons and Blumstein 2010a, b) and European red foxes (Ritchie and Johnson 2009, Wallach et al. 2009). For the aged urine, we selected a batch of urine that had previously deterred western gray kangaroos (Parsons and Blumstein 2010a). Very small volumes had been drawn from this batch, and we have worked directly with this particular lot in both laboratory and field conditions. Forty milliliters of each treatment was added to 1.5 g of hygroscopic crystals (Hortico<sup> $\mathbb{R}$ </sup> water storage crystals) and placed in clean Petri dishes. Water-saving crystals are normally used in horticultural applications to bind and preserve water. In our experiment, the crystals were employed to solidify treatments so it could withstand rainy conditions, slow evaporation, protect against fouling the area in case of spillage, and minimize researcher influence on the experiment.

We provided one treatment every 3 days, haphazardly presented over 18 days (Rittschof and Hazlett 1997). Each treatment was evaluated for 6 12-hour periods (dusk to dawn).

#### **Statistical Analyses**

Nonnormal data were log-transformed prior to analyses. We fitted MANOVA (MINITAB v 14.1, Chicago, IL) models to explain variation in all response variables. We examined the number of video captures of western gray kangaroos and foxes during each dusk:dawn period (1800 hours through 0600 hours) over 18 evenings. Additionally, we considered the number of approaches and vigilance-flight from the treatments (Parsons and Blumstein 2010a, b). For foxes, we also scored the number of "attractions" to a treatment. An attraction was considered as  $\geq 3$  repeated attempts to approach the treatment. We used Tukey's B post hoc analysis for pairwise comparisons. We counted the number of false triggers and instances that kangaroos were "heard" but not directly observed during the video. This information was omitted from analyses when no association was observed with any treatment.

## RESULTS

Compared with our distilled water control, both dilute methanol and aged dingo urine proved biologically inert, and did not influence vigilance or aversive behaviors among

**Table 1.** Behavioural responses from western gray kangaroos (*Macropus fuliginosus*) and European foxes (*Vulpes vulpes*), at Herne Hill, Western Australia to potential matrices for a synthetic dingo urine. Captures are defined as a target animal recorded in the vicinity; approaches are defined as a target animal investigating the scent to within 1 m; vigilance is defined as animals scanning beyond visual reach with ears erect. Treatments included distilled water, 2007 aged dingo urine (*Canis lupus dingo*), and 10% methanol. Means are provided as daily raw counts  $\pm$  standard error.

Treatment	Distilled water	Old urine	10% Methanol	Significance (MANOVA)
Kangaroos				
Captures	$7.33 \pm 1.91$	$8.66 \pm 1.532$	$10.40 \pm 2.40$	$F_{2,17} = 0.69; P = 0.515$
Approaches	$1.50\pm0.80$	$3.00 \pm 0.756$	$1.60\pm1.60$	$F_{2,17} = 0.93; P = 0.417$
Vigilance	$0.00\pm0.00$	$0.143 \pm 0.143$	$0.00\pm0.00$	$F_{2,17} = 0.76; P = 0.483$
Foxes				
Captures	$0.667 \pm 0.667$	$1.857\pm0.595$	$0.600 \pm 0.245$	$F_{2,17} = 3.72; P = 0.049$
Approaches	$0.00\pm0.00$	$1.143 \pm 0.340$	$0.200 \pm 0.200$	$F_{2,17} = 6.23; P = 0.011$
Attraction	$0.00\pm0.00$	$0.857\pm0.261$	$0.00\pm0.00$	$F_{2,17} = 8.25; P = 0.004$

kangaroos (Table 1; Fig. 2). Foxes, which visited almost nightly during the trial, were also not aversively impacted by either of the matrices. However, foxes were more likely to approach the 2007 (aged) dingo urine, than the dilute methanol or the distilled water (MANOVA:  $F_{2,17} = 6.23$ ; P = 0.011). Foxes did not increase their vigilance or startle in response to the aged urine (Fig. 3); however, foxes were highly attracted to the aged urine as compared with methanol and the control (MANOVA:  $F_{2,17} = 8.25$ ; P = 0.004) and were often observed scent-rolling in the treatment.

### DISCUSSION

The use of an in situ bioassay helped us identify a behaviorally benign solvent matrix, dilute methanol, as a possible vehicle to support the mixing and presentation of deterrent infochemicals in free-ranging conditions. It came as little surprise that aged dingo urine, a previously active signal, was ignored by kangaroos because we have observed that degraded (aged) urine has an attenuated effect (Parsons and Blumstein 2010*a*). This lends support to our concerns that a lack of response to a predator signal—over time—may be falsely ascribed to habituation. The actual reason for the decreased response may be due to the constantly degrading (aged) signal, as a consequence of the differential volatility among the active constituents in the compound (Parsons and Blumstein 2010*b*). Together, this provides affirmation that appropriate (non-

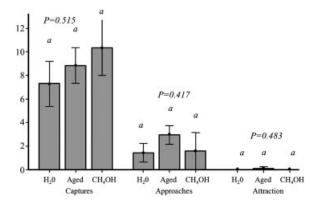
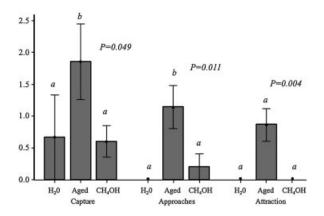


Figure 2. The number of captures (left), approaches (center), and vigilance (right)  $\times$  treatment ( $\pm$ SE) for western gray kangaroos exposed to 10% methanol, aged dingo urine, and distilled water in Herne Hill, Australia, May 2010. Different superscript letters differ significantly by Tukey's *B*-test.

interfering) matrices should be investigated to resuspend as many active chemicals as possible.

Surprisingly, the old urine did not generate any more approaches by kangaroos than the distilled water control. We previously hypothesized that aged dingo urine should attract kangaroos because animals (in theory) should inspect the urine for biologically relevant information, such as a possible time stamp, or level of hunger of the predator (Wyatt 2003). However, following these trials, we have restructured our interpretation to consider that there might be a limit to the age of urine, beyond which kangaroos will not bother to approach or investigate the scent.

Conversely, we were surprised that foxes were attracted to investigate the aged urine, without demonstrating vigilance or startle. This behavior is in direct contrast to foxes' adverse responses to urine collected in March 2010 (M. H. Parsons, unpublished data). It is not clear what value an aged scent would represent to foxes scent-rolling in it. Animals sometimes don the scent of a predator to make themselves more "scary," or less apparent, to other potential predators (Clucas et al. 2008). However, the answer might be less complicated; the structure of kairomones is quite similar throughout the animal kingdom (Wyatt 2003), and one taxon may be inadvertently affected by chemicals from another. In this light, foxes may not have recognized the degraded dingo scent as such.



**Figure 3.** The number of captures (left), approaches (center), and attraction (right)  $\times$  treatment for European foxes exposed to 10% methanol, aged dingo urine, and distilled water in Herne Hill, Australia, May 2010. Different superscript letters differ significantly by Tukey's *B*-test.

One commonly reported experimental flaw, "high feeding motivation," has the potential to reduce confidence in repellent results, because the interaction of opposing scents from the attractive fodder, in contrast to the potentially repellent treatments, may confound interpretation of results (Kimball et al. 2009). We have increased confidence in our results using this design, because the fence-crossing area was located at a sufficient distance (>50 m) from the attractive food source.

## MANAGEMENT IMPLICATIONS

Partial synthetics have provided mixed results and composite synthetic urines have not previously been created. If land managers and conservationists are to accurately synthesize and present predator signals as noninvasive agents for animal management, they will be required to add active info-chemicals to slightly polar solvents that serve as a backbone matrix for the compound. Researchers will also be required to implement new bioassays to humanely evaluate the behavioral responses of target animals in free-ranging environments. Our bioassay was sensitive enough to allow us to differentiate among scents that have limited effect on target species behavior. We have found that dilute methanol, a natural substance, may serve as an appropriate matrix for synthetic dingo urine. The aged dingo scent, however, attracted foxes to the area, limiting usefulness of this type of compound in the production of deterrents. Additional carriers should be investigated, though these putative agents should co-occur naturally with target and nontarget species if we are to minimize the potential for neophobic effects.

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