Biologically meaningful scents: a framework for understanding predator–prey research across disciplines

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ABSTRACT

Fear of predation is a universal motivator. Because predators hunt using stealth and surprise, there is a widespread ability among prey to assess risk from chemical information – scents – in their environment. Consequently, scents often act as particularly strong modulators of memory and emotions. Recent advances in ecological research and analytical technology are leading to novel ways to use this chemical information to create effective attractants, repellents and anti-anxiolytic compounds for wildlife managers, conservation biologists and health practitioners. However, there is extensive variation in the design, results, and interpretation of studies of olfactory-based risk discrimination. To understand the highly variable literature in this area, we adopt a multi-disciplinary approach and synthesize the latest findings from neurobiology, chemical ecology, and ethology to propose a contemporary framework that accounts for such disparate factors as the time-limited stability of chemicals, highly canalized mechanisms that influence prey responses, and the context within which these scents are detected (e.g. availability of alternative resources, perceived shelter, and ambient physical parameters). This framework helps to account for the wide range of reported responses by prey to predator scents, and explains, paradoxically, how the same individual predator scent can be interpreted as either safe or dangerous to a prey animal depending on how, when and where the cue was deposited. We provide a hypothetical example to illustrate the most common factors that influence how a predator scent (from dingoes, *Canis dingo*) may both attract and repel the same target organism (kangaroos, *Macropus* spp.). This framework identifies the catalysts that enable dynamic scents, odours or odorants to be used as attractants as well as deterrents. Because effective scent tools often relate to traumatic memories (fear and/or anxiety) that cause future avoidance, this information may also guide the development of appeasement, enrichment and anti-anxiolytic compounds, and help explain the observed variation in post-traumatic-related behaviours (including post-traumatic stress disorder, PTSD) among diverse terrestrial taxa, including humans.

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I. INTRODUCTION

Individuals that leave the most descendants may do so because they effectively and efficiently trade-off the assessment and management of predation risk with other important activities. Olfactory information often plays a central role in assessment of risk because both predators and prey produce general body odours derived from skin secretions modified by microbial activity (Davis *et al.*, 2013), with contributions from dietary compounds and their metabolism (Ferkin *et al.*, 1997), as well as odorants from excreta and specific glandular secretions, including steroid hormones (Zamaratskaia & Squires, 2009). Some of these chemicals are incidental to general metabolism, while others have evolved to provide signalling functions. Our knowledge of these potential signals and the trade-offs that individuals make in response to them provides a framework with which we can understand risk management behaviours for a variety of organisms.

Indeed, olfaction is an ancient, but key modality for diverse groups of terrestrial taxa from insects (Persons & Rypstra, 2001) to primates (Willems & Hill, 2009). Because the original, ancestral mammals were probably nocturnal and used their olfactory abilities to help locate resources and keep safe (Eisenberg & Eisenberg, 1981), we should expect that olfactory stimuli may have profound effects on many mammals, including humans. Olfactory–limbic pathways are more direct than other senses (visual, tactile, acoustic), synapsing directly with the amygdala–hippocampal complex (Otto *et al.*, 1991; Dusek & Eichenbaum, 1998). Odour-evoked memories often are more vivid and arousing than auditory (Toffolo, Smeets & Van Den Hout, 2012) or visual memories (Herz, 2004). Thus, odours are particularly effective at triggering aversive memories associated with stress or trauma (Wiemers, Sauvage & Wolf, 2014).

By studying olfactory-mediated predator–prey interactions we gain new insights and strategies to create more effective wildlife lures (Andelt & Woolley, 1996) and wildlife deterrents (Schulte, 2016), as well as tools to enrich the lives of captive animals (Wells & Egli, 2004). These insights are not restricted to non-humans. For humans, these insights allow us to create novel tools to reinforce emotional states (Clark & King, 2008), reduce anxiety (Sheppard & Mills, 2003), create new models for human pathophysiology (Cohen *et al.*, 2006; Mackenzie *et al.*, 2010; Clinchy *et al.*, 2011), and develop olfactory applications for military use

(Schmeisser, Pollard & Letowski, 2013). While there are many promising avenues for this research, the first few decades of predator–prey research have presented many challenges and more questions than answers.

Over a decade ago Apfelbach *et al.* (2005) synthesized the first few decades of research involving animal-derived chemicals and their impacts on potential prey. They attempted to account for a wide variety of prey responses that included apparently contradictory responses. At one end of the response spectrum, prey may ignore a predator scent (Orrock & Danielson, 2009), express mild inhibitory or repellent activities (Herman & Valone, 2000), or suppress non-defensive behaviours (feeding, resting; Herman & Valone, 2000). At the other end of the response spectrum, animals may substantially modify their diurnal activity patterns (Fenn & Macdonald, 1995) and habitat use, generating what are perceived as 'area effects' where an animal is deterred for some appreciable distance (Shrader *et al.,* 2008; Willems & Hill, 2009; Parsons & Blumstein, 2010*a*; Cox *et al.,* 2012). Additionally, while animals are well known to habituate to predator signals by showing a decreased response over time (Bytheway, Carthey & Banks, 2013), in some cases animals may show the opposite effect and become sensitized and thus more responsive to aversive stimuli over time (Parsons & Blumstein, 2010*a*; Götz & Janik, 2011; Cox *et al.,* 2012).

Importantly, Apfelbach *et al.* (2005) helped focus research by heightening our awareness of the neurobiological basis for interpreting scent responses amidst the early questions in the field. Such questions included: whether predator scents or odours are interpreted by prey as fearsome, how learning (previous exposure to a scent; Blumstein *et al.,* 2002) modifies their response, and whether the behavioural response is generated *via* non-fear-based mechanisms such as decreased food palatability (Kimball & Nolte, 2006). Additionally, these researchers emphasized the importance of the source of each cue, because scents from urine, faeces, dander, tears, saliva, sebum, porphyrin fur, feathers, scales, and cutaneous gland secretions can elicit different responses by prey.

Now, in the second decade of the 21st century, we have improved analytical capabilities (Schmeisser *et al.,* 2013) and sampling protocols (Parker, 2010). These innovations have helped us to understand that scents may contain more specific information than was previously recognized. An olfactory secretion may provide information about an individual's age and social class (Osada *et al.,* 2008), individual identity and genotype (Kerley & Salkina, 2007), health (Zala, Potts & Penn, 2004), disease status (Lanuza *et al.,* 2014; Olsson *et al.,* 2014), hunger level and diet (Wyatt, 2010) as well as additional information being conveyed from patterns of marking and overmarking (Banks, Daly & Bytheway, 2016). This information is available to conspecifics, as well as being potentially available to heterospecifics (Hughes, Korpimäki & Banks, 2010*a*; Hughes, Price & Banks, 2010*b*; Jones *et al.,* 2016).

In this review, we discuss scent and odour properties of both predator and prey to understand how olfaction works,

as we elaborate on how scents decay, and how decomposition may rapidly modify the potential information content of scent due to the differential volatility of decomposing constituents (Wilder *et al.,* 2005). We discuss how heterospecific animals may interpret this variable information and note that the absence of some molecules may have as much meaning as those that remain (Apps, 2013; Jordan *et al.,* 2013). We also address a number of contextual variables that provide potential information to explain variation in response. These include a variety of indirect environmental cues of risk, such as variation in visibility (Orrock & Danielson, 2009) and the vulnerability of prey without shelter (Busch & Burroni, 2015). Understanding the variety of potential information in a scent is essential if we intend to synthesize or remix complex scent compounds to mimic the original scent accurately.

(1) Objectives

We follow several goals in this review. First, we provide a 10-year update to the Apfelbach *et al.* (2005) seminal paper on predator scents and prey responses. We discuss the literature in the context of recent trends in neurobiology, chemistry, and ethology while addressing some of the puzzling questions and conundrums that have been revealed since that paper was published. Second, as we develop a multi-disciplinary framework, we consider insights from these three disciplines as 'catalysts' and discuss how perplexing questions that arise within each catalyst can be accounted for against a range of possible outcomes. Third, we highlight challenges in the process of creating effective scent-based tools, and reinforce the notion that post-production management of these tools must explicitly account for the contextual conditions that most often promote success. While our focus is primarily to explain variability in the nature of responses by prey to predator scents – specifically with respect to creating attractants and repellents that can be used in wildlife management and conservation biology – our framework also provides information on other practical uses of scent including zoo-enrichment scents (Fay & Miller, 2015; Samuelson *et al.,* 2016) appeasement and anti-anxiolytic pheromones (Landsberg *et al.,* 2015; Osella *et al.,* 2015), and other behavioural-modification cues to influence the mood or emotional state of many terrestrial animals (DePorter, 2015), including humans.

II. NEUROBIOLOGICAL MECHANISMS UNDERLYING PREY RESPONSES

At any given time, the environment contains a vast array of substances that can be detected by chemical-sensing receptors. Airborne volatiles readily access olfactory receptors *via* inspired air, while non-volatile chemicals can be chemically sensed when in aqueous solution (Brennan & Kendrick, 2006). For example, mice make direct nasal contact with urinary scent marks, enabling non-volatile proteins and their ligand complexes to be taken up by
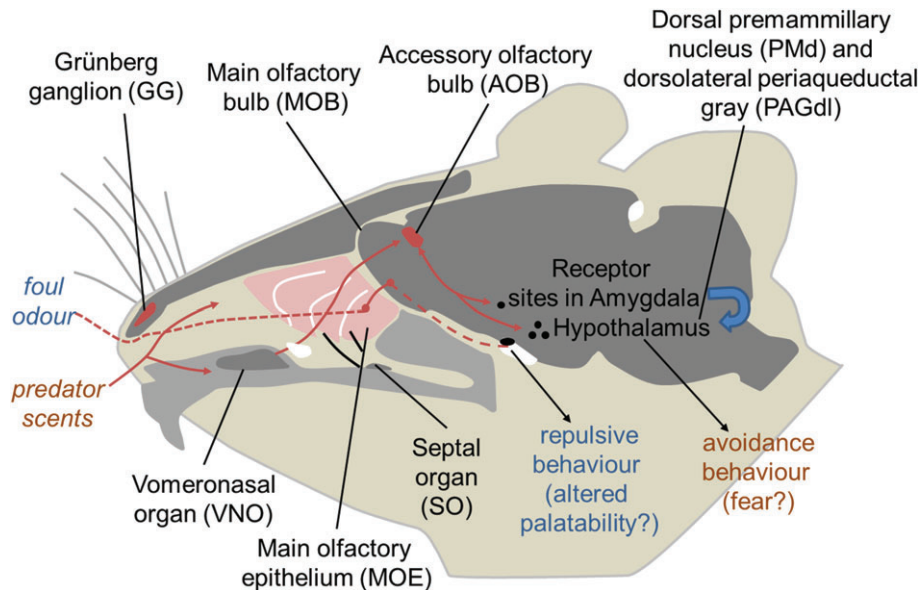
Fig. 1. The chemo-sensory structures involved in scent and odour-based avoidance. The vomeronasal (VNO) pathway of a scent is shown by a solid line and the main olfactory pathway of a foul odour is shown by a dotted line.

the vomeronasal organ (Hurst, 2009). The challenge for the chemosensing individual is to first detect and then disambiguate biologically meaningful information from irrelevant signals and background noise, and finally to interpret and respond to it. A prey animal may respond by becoming attracted to the scent (positive valence), ignoring it (0 valence), or by exhibiting a behavioural aversion to it (negative valence). A deep appreciation of the mechanisms by which prey species accomplish this task (e.g. the neuroanatomy of the prey and how they physiologically detect and assess risk) is essential for the proper interpretation of predator–prey interactions.

(1) Anatomy of olfactory circuits

It used to be thought that most mammals have two olfactory systems working independently to detect chemicals: the primary olfactory epithelial tissue (main olfactory epithelium, MOE) and the vomeronasal organ (VNO; Fig. 1). The MOE was long regarded as the key structure that received information about the chemical environment, including odours emitted by predators. The signals received by the olfactory epithelium are transmitted to the olfactory bulb (bulbus olfactorius, BO), and from there to higher brain centres. The VNO in turn was assumed to be the receiving station for intraspecific signals such as pheromones, which would then be transmitted to the accessory olfactory bulb (AOB), and subsequently to higher brain centres.

We now recognize that sensory neurons in both the MOE and VNO are activated by general and pheromonal odorants, and that fear and avoidance behaviours are often associated with the AOB (Blanchard, Defensor & Blanchard, 2010; Canteras, Pavesi & Carobrez, 2015). This would imply – at least amongst among the rodent species in which

this has been studied (Ben-Shaul *et al.*, 2010; Takahashi, 2015) – that predator scents could be detected by the same pathway as pheromones. Indeed, in a recent study with golden hamsters (*Mesocricetus auratus*), removal of the VNO completely impaired the defensive response to predator odour (Zhao & Liu, 2015). It remains unclear however, what process initiates sampling by the VNO. Combined behavioural and gene activation studies (specifically of the gene *c-Fos*) in mice (Slotnick *et al.*, 2010) support the hypothesis that odour detection by the MOE is required to initiate sampling by the VNO.

Both subsystems converge in the brain in the medial amygdala and ventromedial hypothalamus – structures involved in the inducence of fear, anxiety, and defensive behaviours (for a detailed review, see Kelliher & Wersinger, 2009). The medial zone of the hypothalamus is often referred to as the 'defensive response circuit', and through the use of conditioning treatments, researchers can even cause neutral odours to be associated with anxiety or trauma (Canteras *et al.*, 2015). Among rats and mice, this region has a prominent dorsal premammillary nucleus (PMd) and dorsolateral periaqueductal gray (PAGdl). These nuclei are key sites that influence fear and contextual conditioning. The PAGdl may be particularly important to the flight or escape response (Fogaça *et al.*, 2012). PMd and PAGdl are also thought to be amplifiers of the defence circuit, and any lesions in this area are directly responsible for reduced anti-predator responses (Blanchard *et al.*, 2010).

Additional chemo-sensitive structures, or accessory olfactory organs, also exist. The functions of these structures (Canteras *et al.*, 2015; Fig. 1) are still not fully understood, although each is considered below in the context of understanding prey responses to predator scents or odours.

(2) Accessory structures

The septal organ (SO), also called the organ of Masera (Broman, 1921), is an isolated patch of olfactory–sensory epithelium located bilaterally at the ventral part of the mammalian nasal septum and separated from the MOE by a region of modified respiratory epithelium. Identified initially in newborn mice (*Mus musculus*), the SO was described subsequently in several other mammalian species (for review, see Farbman, 1992). A possible function of the SO is in sensing chemicals from fluids entering the mouth by licking. Like the VNO, this region may be important in regulating social/sexual behaviour, but appears to exist only in species where the nasopalatine duct (NPAL) does not open into the VNO (Weiler & Farbman, 2003). It is not expected that this region plays a prominent role in anti-predator responses, although it is possible.

The Grüneberg ganglion (Grüneberg, 1973) is also an anatomically distinct organ, and is implicated in the detection of two types of volatile 'danger' compounds: alarm pheromones emitted by stressed conspecifics, and kairomones emitted by carnivores (Brechbühl *et al.*, 2015). It is thought that many fear-based responses, especially in rodents, are generated in this region, but research to date has been scant. Solitary chemosensory cells (SCCs) are isolated elements typically found in the skin of aquatic vertebrates. Recently, these cells were also found in internal organs of mammals (Sbarbati & Osculati, 2003; Lin *et al.*, 2008). The SCCs of aquatic vertebrates play a role in detecting food and predators. The functional role of SCCs in mammals is largely unknown; however, they do respond to odorous irritants, indicating that the SCC may be involved in some inflammatory or tactile responses to scent.

The oral cavity is also involved in scent perception because scents may alter gustatory sensations or palatability. Field experiments commonly employ food trays that are placed within the vicinity of a predator scent and left overnight, with the leftover food (referred to as giving up density or GUD) measured as a response variable to the scent treatment (Carthey & Banks, 2014). Sometimes this forfeiture of food is conflated with a fear response (Kimball & Nolte, 2006) (Fig. 1), even though animals may generally consume less food when it is placed near foul-smelling scents. For example domestic sheep (*Ovis aries*) forage less when their food is placed near pig (*Sus scrofa*) or other sheep faeces (Arnould & Signoret, 1993), cattle (*Bos taurus*) avoid feeding near their own faeces (Dohi, Yamada & Entsu, 1991), and goats (*Capra hircus*) avoid feeding near cattle faeces (Aoyama *et al.*, 1994). These responses are less related to fear, and more to repulsion, an adaptive response to minimize risk of disease (Hutchings *et al.*, 1998; Ezenwa, 2004). Although clear in these situations, we later show how these very different responses can be disambiguated by carefully selecting response variables.

(3) Recognition and olfactory learning

Responses to olfactory stimuli may either be highly canalized and influenced by a specific gene, or require some prior experience. A laboratory rat's (*Rattus norvegicus*) defensive response to cat (*Felis catus*) odour may be performed competently with no previous experience of a cat. Some researchers refer to this example as 'species memory', 'phyletic memory' or 'innate response' (Canteras *et al.*, 2015). Other researchers avoid using the term 'innate', partly because of the complexity of the response, and also because of the role of learning in the response, even prior to birth in some cases (Doty, 2012). Whether scents invoke innate responses thus remains debated in the literature, but it is clear that some organisms do respond to predator scents on first exposure.

Scent-recognition by mammalian prey is complex and may be influenced by imprinting-like learning processes that occur as early as 60–90 days postnatal in black-footed ferrets (*Mustela nigripes*; Vargas & Anderson, 1996), and European ferrets (*Mustela putorius f. furo*; Apfelbach & Kruska, 1979; Apfelbach, 1986). Less is known about how prey learn to respond to predators. Monclús *et al.* (2005) found that predator-naïve European rabbits (*Oryctolagus cuniculus*) responded behaviourally and physiologically to predatory fox (*Vulpes vulpes*) scents. A more recent study of mice (*Mus musculus*; Pérez-Gómez *et al.*, 2015) showed that experience is not necessary to govern a response. Similarly, black-tailed deer (*Odocoileus hemionus columbianus*) can discriminate between the scent of dangerous predators (wolves, *Canis lupus*) that have been absent for over 100 years and cues from less-dangerous predators (black bear, *Ursus americanos*) that are currently present (Chamaillé-Jammes *et al.*, 2014). Another study (Osada, Miyazono & Kashiwayanagi, 2014) showed that Hokkaido deer (*Cervus nippon*) would respond to scents of Japanese wolves (*Canis lupus hodophilax*), even though this predator had been extinct locally for about 100 years. Both studies seem to indicate that any phyletic knowledge might persist for long periods of time.

It is costly for an organism to respond to predators that are extinct, therefore we should expect behavioural changes among prey to be rapid in the absence of predation (Lahti *et al.*, 2009). After all, it is not in an animal's best interest to be overly cautious in environments under decreasing predator threat (Hollings *et al.*, 2015). This assumption may partly explain why some prey responses to new predators are often learned during their development (Blumstein *et al.*, 2002; Anson & Dickman, 2013; Carthey & Banks, 2015; Tortosa *et al.*, 2015). And learning certainly influences the degree of responsiveness by predator-naïve rabbits (Rödel, Monclús & von Holst, 2006). On the other hand, behavioural and other prey responses to predators may persist despite the extinction of a specific predator (Blumstein, 2006). We caution however, that heightened aversion to a particular scent does not necessarily mean that the prey species recognizes the scent from a specific predator *per se*.

(4) Conflicting roles of habituation and sensitization

A dual-process theory may be used to describe changes in response from repeated exposure to a negative stimulus

like a predator scent. Groves & Thompson (1970) referred to the processes as either decremental or instrumental. Habituation – a decreased response over time – is inferred from decremental changes, especially where animals are assumed to learn that a fear cue is unaccompanied by risk and ignore it (Powell & Banks, 2004; Cox *et al.*, 2010), Habituation is commonly reported as an outcome to scent exposure in the predator–prey literature (Apfelbach *et al.*, 2005). However, we also refer the reader to an alternative explanation for decreased responsiveness in Section III.4.

In other studies, the opposite response has been recorded. Instrumental changes (animals becoming more responsive through sensitization) were shown by Götz & Janik (2011), who found that phocid seals (*Halichoerus grypus* and *Phoca vitulina*) became more responsive to acoustic threat stimuli over time (Götz & Janik, 2011, 2015). Similarly, possible sensitization has also been reported among terrestrial eutherian mammals (Cox *et al.*, 2012) and among marsupials presented with olfactory predatory scents (Parsons & Blumstein, 2010*a*). In order to create more-effective deterrents, managers may need to recreate the conditions under which sensitization is most likely to occur. We discuss a few of the contextual conditions that may facilitate this in Section V.

III. CHEMICAL INFORMATION SYSTEMS

In the predator–prey context, each actor has an interest in obtaining information about the presence and activities of the other or others. There are at least three mechanisms whereby scent may provide information: *via* the chemicals in the secreted compound, the space and time component of the deposition, and by over-marking of the signals (e.g. an individual deposits its own mark over scents previously deposited by other animals) forming a complex mixture (Hurst & Beynon, 2004). In each case, these are complex scents, and it must be understood that composite signals, not unlike gene sequences, may contain 'junk information' that is not informative (Apps, Mmualefe & McNutt, 2013), while fine differences between scents may convey essential information about risk and opportunity to both conspecifics and heterospecifics.

(1) Conspecific communication

Group-living mammals and social predators that need to broadcast information over distance may use scents to communicate a wealth of information to conspecifics. The mixture of odorants in body odour and scents from urine or specialized secretory glands is highly specific. For instance, mouse (*Mus musculus*) urine contains major urinary proteins (MUPs; Zhou & Rui, 2010) which reflect the mouse's major histocompatibility complex (MHC) haplotype, and these are sufficiently variable to convey information about sex, social status and clan (Hurst, 2009). Scents often contain information about the social, sexual, nutritional, health and reproductive status and microbial flora of each individual (Hurst & Beynon, 2004; Wyatt, 2010).

When more than one individual over-marks or scent-blends, this complex mixture may identify groups instead of individuals (Vogt *et al.*, 2014). Such chemical 'bulletin boards' can be used by males to advertise availability, resources and their presence, while females may advertise their reproductive status. This information is used by many species of terrestrial mammals, and such bulletin boards are diverse in structure, composed of urine, faeces, para-anal (hyenas, *Crocuta crocuta* and *Hyaena hyaena*; Theis, 2008) or para-cloacal gland deposits (marsupial carnivores; Ruibal, Peakall & Claridge, 2011), and cheek gland secretions (felids, viverrids; Moran & Sorensen, 1986). These advertisements may be deployed in latrines (Tasmanian devils, *Sarcophilus harrisii*), scratching trees (felids) or even deposited on stems of grass (hyenas).

House mouse 'scent wars' are elaborate. Whenever a conspecific leaves a scent mark, territory owners rapidly countermark and replenish marks at a higher rate than usual. This information not only shows current dominance, but due to the most stable molecules (MUPs) secreted in the urine, provides a record of past challenges to the dominant male (Hurst & Beynon, 2004). In the process, these bulletin boards also provide a record for heterospecifics to obtain information about their competitors and predators (Banks *et al.*, 2016). Indeed, the volatile odour profile is an open broadcast system that could directly and indirectly influence communities and trophic networks (Hughes, Kelley & Banks, 2012; Jones *et al.*, 2016).

(2) Heterospecific information within scents

More experimental evidence is needed to determine the extent of information that heterospecifics can extract from scents of other species, whether these cues are from predators, prey, competitors or even alarm cues from other species. Dogs (*Canis familiaris*) can discriminate individuality among humans from hand scents, even after perfume has been applied (Curran, Prada & Furton, 2010). This ability is not limited to domestic animals. Tasmanian devils, when trapped, can differentiate between individual human handlers (M. Jones, personal observations).

The lack of additional evidence showing heterospecific scent discrimination abilities may be partly because existing biological assays have focused primarily on utilizing 'representative' scents from predators, without regard to testing variations among scents present within a predator species. Such variation could arise between, for example, alpha predator scents and those from subdominant males, females, or juveniles; the risks conveyed from each scent could potentially differ. Certainly, animals are physiologically capable of receiving heterospecific scents and some species (such as dogs and Tasmanian devils) are able clearly to differentiate individual scents of other species (humans). This point becomes extremely important when we discuss the importance of creating accurate synthetic analogues, or mimics, for use as scent tools.

(3) Environmental persistence and decay of scents

We are only just beginning to appreciate how the chemical structure, plasticity and stability of chemical cues (scent marks) interact over space and time with physical parameters of the environment. This is true also for understanding how these factors influence the intended and non-intended messages sent to target and non-target organisms. Rain, ultraviolet (UV) radiation, and bacterial decomposition degrade or otherwise modify scents (Muller-Schwarze, 2006). Pheromones can also be oxidized, hydrolysed, or destroyed by UV light. For example, the (Z)-7-12-acetyl derivative in elephant urine will gradually hydrolyse (Rasmussen, 1988), with its lipoprotein carriers possibly determining the lifetime of the signal. Lipoproteins play another role in that they filter and select odorants, confer specificity, and play a critical role in the transport and transfer of an active ligand to the vomeronasal organ of the receiver (Rasmussen & Schulte, 1998).

Scent marks consist of multiple compounds of varying volatility, with the extremes varying from highly volatile to non-volatile. The latter include proteins, lipids and high molecular weight hydrocarbons such as squalene. The non-volatiles may function as 'keeper substances', modulating evaporation rates of the volatiles (Muller-Schwarze, 1989). Some non-volatile components of scents are so stable they must be released by the receivers, which either exhale on, lick, or urinate on the scent mark (Alberts, 1992). From studies of the physicochemistry of mixtures of volatile liquids, we also know that the vapour pressure of each component is reduced by its molar fraction in the solution, with deviations where there are attractive interactions between the molecules of each (Raoult's and Henry's Laws; Sinko, 2006). The lipids in many scent marks will lower the vapour pressure of volatile constituents, slow down their evaporation rate and thus render the scent marks more persistent. Direct evidence of this effect comes from studies of the release of phenylacetic acid, an active compound in the scent mark of the Mongolian gerbil (Meriones unguiculatus) (Regnier & Goodwin, 1977). Both sebum and squalene, common integumental lipids, slowed the volatilization of phenylacetic acid. However, the surface area and chemical nature of the substrate is also important. Porous materials, such as clay or wood, retained scents much longer than surfaces such as smooth rocks or metal. Consequently, the substrate a chemical is placed on should be carefully considered during experimental planning and during the deployment of attractants or repellents.

The rate of chemical release can be accelerated by higher humidity, as water and volatile organic compounds compete for surface sites. Increased temperature will increase vapour pressure, resulting in a faster release of volatiles (Muller-Schwarze, 2006). Another type of temperature effect is known and has been demonstrated in the femoral secretion from male wall lizards (Podarcis muralis). Males on warmer basking sites – a valuable resource – produce a different secretion (chemical signature) than do males on cooler basking sites, the former being richer in compounds that are thought to play a role in male–male interactions and female choice (Heathcote et al., 2014). This may be the first report of thermal plasticity in a vertebrate's chemical signature. The very act of freezing pheromones during experimental preparation can also lead to degradation. This is seen in how male mice respond differently to female urine (Hoffmann, Musolf & Penn, 2009), and how Brandt's voles (Lasiopodomys brandtii) respond differently to fresh cat faeces and those frozen at −70°C (Hegab et al., 2014). We will revisit this important topic in more detail in Sections IV and V.

(4) Consequences of decay: signal intensity, duration and meaning

We previously defined habituation as a reduced response to a stimulus over time, and this may occur when an animal learns that a risk cue is unaccompanied by danger. Yet, scent marks also are modified – along with their initial biological meaning – over time, due to chemical decomposition and by the evaporation of volatiles (Fig. 2). Thus, in at least some cases of presumed habituation, it is suspected that the causal mechanism may be due to the changed chemical structure and hence information content of a complex scent over time (Parsons & Blumstein, 2010a). In odour mixtures, more-volatile compounds evaporate first. Depending on the role of specific volatile components, the signal may become weaker or communicate to prey that the predator is no longer present.

Animals across many taxa respond differently to scents of varying ages, even when produced by the same species or individual. For instance, cheek gland scents of the African dwarf mongoose (Helogale undulata rufula) are detectable by humans for 1–2 days, while anal gland secretions are noticeable for 10 days (Rasa, 1973). Grey wolves (Canis lupus) regularly re-mark with scents that are strong enough to be detected by humans up to 10 m away. Yet, the scents dissipate quickly, and if wolves do not re-mark within 23 days, the scent marks are assumed to be below detection level because animals no longer over-mark after this time (Peters & Mech, 1975). Brown hyenas (Hyaena brunnea) paste two different secretions on the same blade of grass; a brown mark with an odour that dissipates rapidly (to the human nose), and a white mark that can last up to 6 months (Mills, Gorman & Mills, 1980; Gosling & Roberts, 2001). Similarly, humans can detect urine and scent marks of snow leopards (Panthera uncia) for several months (Jackson & Ahlborn, 1989).

Heterospecifics are apparently as likely to detect changes in age as conspecifics, because this phenomenon is well recognized among organisms as diverse as tadpoles (Peacor, 2006) snails (Turner & Montgomery, 2003), invertebrates (e.g. wolf spiders; Barnes, Persons & Rypstra, 2002) and vertebrates (Parsons, Blumstein & Dods, 2012). There are exceptions. For instance, Bytheway et al. (2013) reported – at least among small mammals (Rattus spp.) – that scent age did not influence response; at least as they measured it. Nonetheless, the temporal effect of scent decomposition
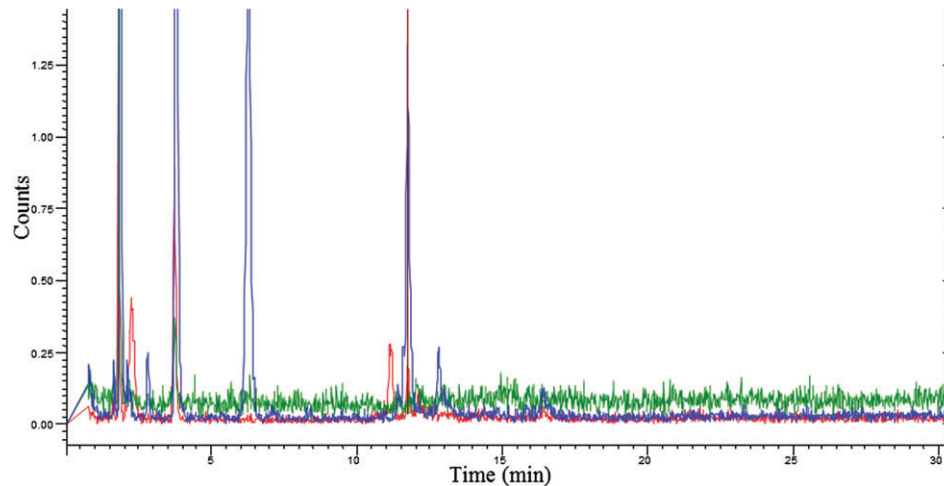
Fig. 2. Profile of new and aged predator (dingo, *Canis dingo*) urine. The blue line refers to fresh male urine, the red line to fresh female urine, and the green line to pooled male and female urine aged >3 months (reproduced with permission from Apfelbach *et al.*, 2015).

Table 1. Glossary

Attractant	A substance that provides incentive for an animal to approach either to obtain a reward, or often, in the case of predator inspection, provides information for risk mitigation
Catalysts	Disciplines, factors or areas of inquiry that may influence outcomes in olfactory-mediated predator–prey studies
Context	The dynamic range of conditions such as the placement of the scent, availability of shelter and alternative resources, that are likely to influence the outcome of an experimental trial
Deterrent	A substance conferring the ability to discourage a target organism from using a specific resource. Usually associated when a sensory stimulus invokes a memory of an aversive event. Not synonymous with repellent
Kairomone	A pheromone that is secreted as an intra-specific cue, but may be detected/interpreted by other species
Macrosmatic	Organisms that evolved to detect and respond to chemical cues as a primary means of predator detection. Humans, in their ancestral state, were macrosmatic
Mechanism of response	The neurobiological chemosensory process by which a scent, odour, or odorant leads to a behavioural response
Pheromone	A chemical cue produced by an organism in order to generate an internal (endocrine) or external response within the same individual or by conspecifics
Scents	A plant or animal-produced (biotic) olfactory cue that may be consciously or subconsciously received by intended, or non-intended species
Odour	A consciously perceived smell that may be produced by biotic or abiotic processes. The sulfurous emanation of a volcano is an example of an abiotically produced odour
Odorants	A subset of constituents that make up a particular odour
Repellent	A substance that causes immediate, usually contact-related (tactile) aversion by a target animal. A capsaicin pepper spray is a repellent

means that the odour will change, the signal will likely be weaker, and it may possibly be detected over shorter distances. This temporal component explains why terrestrial animals, from mice (Garratt *et al.*, 2011) to white rhinoceroses (*Ceratotherium simum*; Owen-Smith, 1971), invest so much time and energy marking and remarking and saturating their walkways while they defend a territory. Both conspecifics (Garratt *et al.*, 2011) and heterospecifics (Jones *et al.*, 2016) may respond to these marks when present and when marks are no longer refreshed. Understanding the potential meaning of this is essential for planning experiments and deploying scent-based tools.

Ultimately, whether a particular scent functions as an attractant or a repellent (to the same organism) depends partly on its concentration (mass/volume) and age. Kairomones (see glossary in Table 1) are said to be 'dose-dependent' or sensitive to concentration (Glimcher, 2010; Vasudevan & Vyas, 2013). By increasing the intensity of an odour, we can enhance, or attenuate its valence (Schmeisser *et al.*, 2013). This point may have been underappreciated in the first few decades of predator–prey field studies, where the need to compare prey responses to multiple predator scents (or predators compared to control herbivores) was seemingly unrecognized. If

the intensity of one predator scent was stronger (more concentrated) than another, then it would have been easy to conclude that the weaker scent conveyed less risk than the stronger (either easier to detect at a longer distance, or more foul) scent.

We have not discussed the importance of diet for determining the concentration of analytes and intensity of the waste cues. However this subject has been reviewed recently (Scherer & Smee, 2016). The authors identified 54 out of 405 studies as showing evidence of prey responding to predator's diet. Some prey responded aversely only after predators had consumed conspecifics as shown in the dwarf hamster (*Phodopus campbelli*; Apfelbach *et al.*, 2015), while other prey used predator diet cues as a secondary source of information to assess risk more accurately.

Given the important effect that decay and diet has on the meaning of the scent, how should one design experiments to study meaning of scents? First, we suggest that properly quantifying scents (e.g. by using an olfactory meter; Fox & Abbott, 2014) can help standardize the intensity (plume) in experiments. Second, we suggest that by using multiple response variables – such as video-recording behavioural responses combined with studies that quantify food consumption – researchers will be better able to extract signal meaning from variable scents (Parsons & Blumstein, 2010*a*; Banks *et al.*, 2014).

IV. PERPLEXING QUESTIONS AND COMMON CONUNDRUMS

Now that we have a better appreciation of the neurobiological mechanisms that underlie an individual's response to scent and the dynamic environmental chemistry that is associated with scents in nature, we can address some common misunderstandings and key issues that confuse interpretations of outcomes in the predator–prey literature.

(1) When the same scent can be an attractant and deterrent: predator inspection

When first detecting a predator or its odour, some prey, seemingly paradoxically, respond by first approaching a potentially fearful scent (suggesting an apparent positive valance) rather than fleeing, freezing, or hiding (negative valence; Zöttl *et al.*, 2012). This phenomenon is called 'predator inspection', and has been reported from a variety of taxa (Fishman, 1999). Approaches (apparent attraction to a deterrent) can indicate that an animal needs to examine an odour or scent more closely to acquire more information about the true risk of predation. Indeed, some solitary felids use a flehmen-like behaviour (suggesting the possible use of the VNO) to investigate novel scents in their environment (Allen, Wallace & Wilmers, 2015). There may be several benefits to approaching a predator scent that include: (*i*) acquiring information about the nature of the potential threat; (*ii*) informing conspecifics of the potential threat; (*iii*)

deterring predator attack; and (*iv*) possibly even advertising one's quality to mates (Dugatkin & Godin, 1992). Finally, animals may interact directly with the scent and liberate molecules that aid in identification and assessment by licking the scent mark or exhaling onto it.

This phenomenon has confounded interpretations; early studies that aimed to show efficacy of deterrents by using the absence of tracks or number of visits to a bait station or feeder, may have unknowingly interpreted these approaches as failures (Banks *et al.*, 2014). Yet, when evaluating the efficacy of a deterrent (Table 1), it is essential to determine whether the same individuals that have been exposed to the scent continue to impinge upon, encroach upon, or consume a protected resource. Even though the same, or different, individuals may continue to inspect a scent – thereby leaving traces of their presence – the deterrent is still 'working' if they retreat without consumption. Sparrow, Parsons & Blumstein (2016) found that southern hairy-nosed wombats (*Lasiorhinus latifrons*) remained within 200 m of dingo (*Canis dingo*) scents throughout a 60-day trial as evidenced by the presence of scat, scratches and tracks. The deterrent was reported to have been 'successful' despite the remaining presence of the target prey animal, because the wombats ceased their problematic digging behaviour and did not re-inhabit any of the collapsed burrows. Had the authors considered 'presence of wombats' as their primary response variable, then this study would have been interpreted, not as a promising success, but as a complete failure. By contrast, the opposite outcome might have been reported if the animals did not revisit a scent once the initial risk assessment had been completed (Banks *et al.*, 2014). Thus, it is essential to understand that a target animal continuing to approach and investigate scents for any 'new' information does not necessarily mean that a deterrent has failed.

(2) When the same scent can be an attractant and deterrent: temporal component

In the previous example, a deterrent functioned briefly as an attractant in that prey inspected the predator cue. In the next example, we consider whether the instability of a degrading cue might also cause a deterrent to become an attractant. Animals may approach a partly degraded scent more closely, and more often, than a fully potent scent, because it is ostensibly more difficult to extract information from an old scent where only the less-volatile components remain, as compared to the scent of a recent void. For instance, wolf spiders (*Pardosa milvina*) have attenuated responses to older predator cues – not from habituation, but from decay – and the scent actually becomes more attractive with age (Barnes *et al.*, 2002). Similarly, western grey kangaroos (*Macropus fuliginosus*) also approach 3-year old dingo scents often enough that they have been evaluated as possible kangaroo attractants (Parsons *et al.*, 2012). Why are animals attracted to degraded scents? One hypothesis is that faded scents may provide information that the signaller is absent (Hurst & Beynon, 2004). Indeed, Jones *et al.* (2016) showed how scents (both their presence and absence) are embedded within information networks, and thus serve indirectly as

both attractants and deterrents to other species in the food web.

(3) Does size matter? Size-structured mismatches between predator–prey pairs

Apfelbach *et al.* (2015) recently suggested that size-structured associations between predators and prey might partly determine how discriminating prey are towards potential information contained in predator scents. Larger prey (lower risk of predation) are expected to be more discriminating between varying predator scents, being most responsive to hungry, healthy, and high-risk predators. We know that ungulates such as elk (*Cervus elaphus*) and bison (*Bison bison*) are more cautious around, and more aggressive towards, high-ranking coyotes (*Canis latrans*) than towards subdominants (Gese, 1999). Such large mammals may benefit from reducing the likelihood of responding by becoming vigilant (a costly behaviour) around all coyotes – even the non-threatening subdominants and juveniles. On the other hand, smaller prey, such as rodents, are fearful of a wider range of predators, because almost any carnivore, large or small, represents a lethal threat.

For instance, the response to scent cues by eastern grey kangaroos (*Macropus giganteus*; a large prey that reaches 1.5 m in height, and weighs 65 kg) to the much smaller Tasmanian devil (12 kg) is revealing. Kangaroos do not respond to devils until devils are fed kangaroo meat (Cox *et al.*, 2010). Findings such as these raise questions as to whether the changes in prey response are due to an 'innate recognition' of conspecifics in the predator wastes, or whether the animals learn to identify the scent of deceased conspecifics in wastes. Regardless, there may generally be size-structured mismatches between the size of the predator and the vulnerability of the prey. This might explain why wombats continued to respond to aged scents placed near their burrows for over 60 days (Sparrow *et al.*, 2016). Wombats depend on their burrows to protect them from predators, and even an aged scent could be risky to this vulnerable prey.

V. FRAMEWORK: MATCHED PAIR EXAMPLE

Below we develop a framework that can be used to understand and predict how a number of factors interact to determine the magnitude (strength or intensity) and direction (positive or negative valence) of a predator–prey scent experiment. This framework is essential if we are to understand the mechanisms by which attractants and deterrents work. We illustrate the utility of this framework by considering the response of kangaroos to dingo scents. Dingo scents are widely reported to trigger vigilant or fearful responses in several marsupials (Parsons & Blumstein, 2010*a*,*b*; Parsons *et al.*, 2012). Yet within our framework, we demonstrate how – from a management perspective – only one of 16 conditions results in true deterrent success whereby dingo scents created an 'area effect' (i.e. the ability to employ a

predator scent to deter a target prey animal some appreciable distance from a guarded food or harborage patch). We emphasize that this worked example should be viewed as a general framework to evaluate a myriad of factors that may influence or confound outcomes and interpretations. There are 16 different (non-mutually exclusive) processes that could occur when an animal encounters a predatory scent.

(1) Catalyst #1: chemistry

In the top section of each panel of Fig. 3 we account for kangaroos' aversion to recently voided dingo scents (Fig. 3A; negative valence, denoted by −) (Parsons & Blumstein, 2010*a*) and their possible attraction to aged scents (Fig. 3B; positive valance, denoted by +) (Parsons *et al.*, 2012). The volatile odour profiles of fresh and aged dingo urine differ significantly (Fig. 2). Thus, in this system, the differential between the stable backbone and most volatile components determine the overall direction of the response (e.g. whether the scent elicits deterrent or attractant behaviours). The state of scent preservation prior to trials is equally important as protein scent-carrier molecules can degrade during storage (Hoffmann *et al.*, 2009). Therefore, a scent may be detected as fearful and thus generate a negative valence (−) if it is a complete, unabridged scent obtained from animals whose scents have been collected and preserved without denaturing the compound. As we move down through the panels we will consider factors commonly affecting the intensity of the response.

(2) Catalyst #2: neuro-mechanism

As we move down through the flow chart for fresh scent deployment (Fig. 3A), we account for how a scent – even when fresh – may elicit highly variable responses from prey animals. A dingo scent with less fidelity, such as after it has been intentionally fractionated in the laboratory (Parsons *et al.*, 2012) or obtained from animals consuming reduced levels of protein (Scherer & Smee, 2016) may evoke a weaker response. Additionally, experimental evidence is beginning to show that scents from adult male African wild dogs (*Lycaon pictus*; Parker, 2010) and dingoes (Fig. 2) are very different to those from females and juveniles. Consequently, scents collected from juveniles or low-ranking males may strongly resemble the odour of dingo to our noses, but may not elicit the same aversive reaction. Rather a kangaroo may still weakly avoid these types of scents while directly under the palate (no valence, denoted by 0); here, the neurological mechanism for avoidance would be altered palatability (Fig. 1), and certainly not fear. It is important to note that for these pungent scents (right side of Fig. 3A) all outcomes will be mildly aversive, at best, for very short distances and only until the smell dissipates.

(3) Catalyst #3: proximity and vulnerability

The factors determining the extent of response to a well-preserved, recently voided, meat-fed, adult male dingo
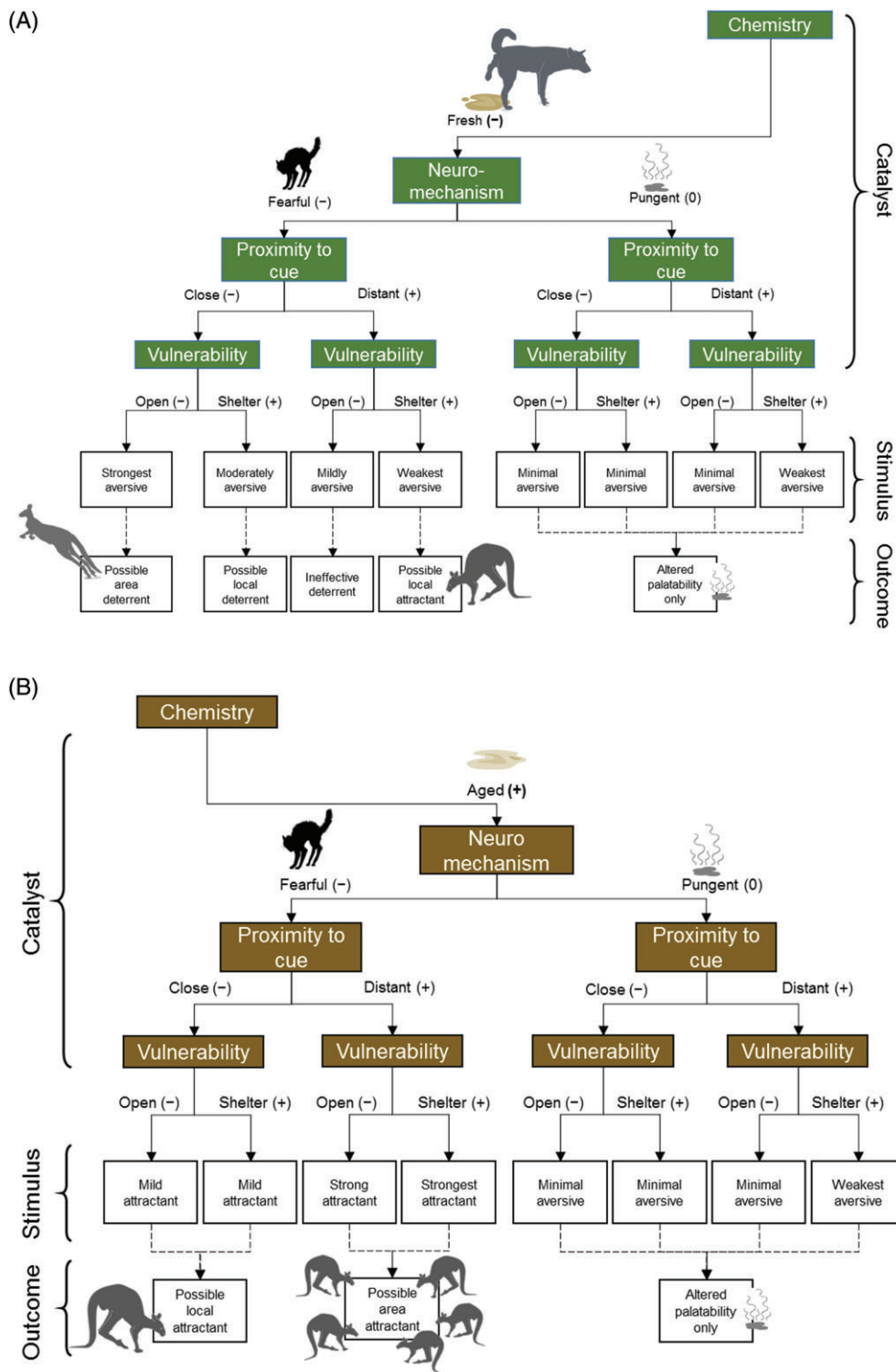
Fig. 3. Some chemical, neurobiological and context-related catalysts that can influence outcomes of olfactory-mediated predator–prey studies. In our example of a kangaroo response to dingo scents, 'fresh' (A) refers to a continually refreshed scent, while 'aged' (B) implies a loss of strength, which often draws animals closer through a process called predator inspection. Valence is indicated by its likely attractant (+) or deterrent (−) properties.

are contextual factors that may relate to the environment within which the scent is deployed. For instance, if a scent is placed within 1 m of a targeted resource (e.g. food or refuge), then it may have an immediate negative valence. Should the scent be deployed further away, say 3–5 m, then there might be a temporary positive valence owing to predator inspection. During this process, a prey animal first approaches the scent (initial positive valence) and evaluates all of the potential information in the scent, before making a decision to ignore the scent, increase vigilance, or potentially avoid the area. The vulnerability of the animal, in Fig. 3A (left side) shown by deployment in sheltered *versus* open field conditions, can determine the magnitude of this response. In our single deterrent 'success', we have a fresh complete scent, deployed close to the targeted resource, in an open area where the target prey animal is most vulnerable, and a strongly aversive startle response is seen in response to scent deposition (Parsons & Blumstein, 2010*a*). In only this one case, among 16 possible outcomes, have we evoked an area effect.

In Fig. 3B, the same set of conditions determines the possible likelihood of a successful attractant. Here the opposite occurs, the most effective attractant scent is based on an aged cue, placed further from the guarded resource, in a sheltered environment where prey animals are less vulnerable and less likely to succumb to predators.

It is immediately apparent that there are more potential catalysts. Among many species, the most important factor might be 'availability of alternative resources'; adding this particular catalyst to our example would make it even more difficult to generate an area effect (now one of 32 successful outcomes). One crude mechanism to estimate how many factors are driving a prey's response might be to examine the number of area effects reported in the literature against the number of failures or very weak effects reported, and to disambiguate (and attempt to reproduce) the specific conditions under which area effects have been reported.

VI. FUTURE DIRECTIONS

New technology has enabled the field of chemical ecology to blossom. This technology can be used both to improve experimental designs using behavioural discrimination assays that allow for a wider range of available behaviours between fine variations in scent, and to create more accurate synthetic analogs that mimic the original scent. We are especially excited to see how this technology will drive a number of improved applications in the fields of wildlife conservation and management, as well as in human health and pathophysiology.

(1) Improved analytical technologies

Our ability to identify fine differences between scents in the field has been enhanced by the creation of mobile analytical capabilities and refined protocols for the detection and characterization of scents (Parker, 2010). Soso *et al.* (2014) coined the term 'ethochemistry' to describe the complementary nature of ethology and chemistry while creating a new sub-discipline that focuses on identifying the potential biologically meaningful information communicated *via* chemical scents. This new field may help address the extent to which scents vary their 'meanings' according to changing ratios of particular elements within compounds (Apps, 2013), or as single molecules that elicit a complete response (Apfelbach *et al.*, 2015). The availability, and soon affordability, of new technology such as higher resolution mobile gas chromatography mass spectrometry (GCMS) units and handheld odour meters, could offer a new level of control in future field and laboratory experiments.

(a) Standardization of scents

There is a compelling need to analyse scents as they are produced and used throughout the duration of observations or experiments. Over the past few decades, previously collected scents have had to be stored prior to field use under low head-space (for decreased evaporation) in cool, dark places to retard degradation between successive trials. These periods ranged from days to months. However, chemical breakdown (particularly the denaturing of protein carrier molecules) occurs even under freezing conditions (Schultz *et al.*, 2000) or when samples are heated or wetted (Wilder *et al.*, 2005).

Some researchers have attempted to work around this scent-decay challenge by collecting and applying new scents as trials proceed (Parsons & Blumstein, 2010*b*; Bytheway *et al.*, 2013). However, this approach too could be problematic; as previously described, successive voids can vary in chemical composition depending on diet (Cox *et al.*, 2010), season, ambient conditions (Wyatt, 2003) or changing social status (Parker, 2010; Fuxjager, Knaebe & Marler, 2015) of the source animals.

A higher level of control is needed to interpret prey's responses to predatory scents. An analogy comes from the field of bioacoustics. When a researcher wishes to determine experimentally whether variable sounds elicit different behavioural responses, they must provide some confidence that the control and treatment signals are broadcast (played back) at the same strength; that is, in this case, the amplitude is constant. A sound pressure-level (SPL) meter is used to ensure that treatment and control sounds are heard by animals at the same volume (at varying distances) from a loudspeaker. The fields of modern pharmacology and reliable drug therapy also rely on the chemical standardization of biologically active substances, which enables the dose–response relationship to be studied in a reliable and predictable manner. Previously, medicines were often natural product mixtures whose potency and effects varied according to their source and storage conditions. Later, pharmacopoeias were established to provide standards by which drugs could be tested.

Until recently, chemical ecologists did not have equivalent hardware to measure the 'intensity of smell' to standardize treatments and controls as in the other aforementioned disciplines. We can now, however, standardize scent treatments and controls by using hand-held 'smell meters' such as the Kanomax OMX-SRM odour meter (Yao *et al.*, 2011; Fox & Abbott, 2014). These units function as the olfactory equivalent to the sound meter, and help quantify differences between scents at each interval of application. Other technologies such as GCMS output, or physical parameters, such as pH and turbidity, can also be used to standardize individual scents before, during, and after trials. For instance, a 6-month trial may require voids collected from a pack of wolves in June, followed by voids each month thereafter. GCMS would identify differences due to changes in diet, health, or social status – all factors that can modify the structure of olfactory signals (Parker, 2010). As technology develops, our understanding and interpretation of the variable literature will grow [a full review of sample analysis and preparation for analytical use is provided by Soso *et al.* (2014)].

(b) Super scents: creating synthetic analogs and novel scent mixtures

When a potentially beneficial scent has been identified, scientists have struggled to move this knowledge from theory into application. Animals do not void wastes often enough for scents to be regularly collected, and this particularly true for endangered predators such as large felids. Some have attempted to harvest scents from captive animals raised specifically to produce scents. However, the very act of moving animals into domestic settings could dramatically change the inherent 'meaning' in the scents (e.g. diet, changes in hormones and social status). There have been several attempts to fractionate scents in order to find a single 'active' component. Apfelbach *et al.* (2005) provided a detailed account of the most common fractions from mustelid, cat and fox odours such as 3,3-dimethyl-1,2-dithiolane (DMDIT), an odour derived from mustelid anal gland secretions, as well as 2,4,5-trimethyl-thiazoline (TMT) n-propylthietane, S-methyl, methyl butanol, isopentyl-methyl sulphide and several others. To our knowledge none of the fractions trialed with larger, ostensibly more discriminating, animals were as remotely effective as the whole compound. In fact, we now know that multiple compounds that differ in concentration and ratio may significantly alter the meaning in each scent variant (Apps *et al.*, 2013).

This too is an area where enhanced GCMS capabilities, now with the ability to detect parts per billion (ppb), can be used. Such assays (e.g. Parker, 2010; Apps *et al.*, 2013; Jordan *et al.*, 2013) should allow determination of accurate mimics with similar ratios and concentrations of mixtures of compounds that work synergistically to convey meaning. The most promising aspect of synthetic scents is their ability to create specific messages. In other words, instead of mimicking a scent that resembles a generic predator odour, we may produce analogs that convey the more meaningful 'hungry, alpha-predator, on a meat-fed diet, in healthy condition'

which conveys enhanced risk and information to potential prey. Thus, synthetic scents may be 'super-scents' that are stronger than the original scent, and also persist longer before degrading. One might, for instance, wish to produce a scent mimic from an overmark sample from a pack of wolves, conveying the message 'many predators'.

(2) New avenues for scent models transcend disciplines: medical and military

There is a variety of well-recognized traditional applications for accurate scent mimics. However, new applications also are emerging. Feline facial pheromones may be used to control cat spraying (Hunthausen, 2000), appeasement pheromones can reduce dog barking (Tod, Brander & Waran, 2005) and potentially limit aggressiveness, while encouraging the activity and habitat use of animals in captive care, such as pinnipeds (Samuelson *et al.*, 2016). Predator scent assays using rats and mice are increasingly used to explore the pathophysiology of humans undergoing post-traumatic stress disorder (PTSD; Clinchy *et al.*, 2011).

This area has received particular interest because PTSD is also the mechanism by which deterrents will work most effectively through sensitization of the target animal (e.g. a scent evokes an unpleasant memory which leads to further anxiety and avoidance). Additionally, although humans have seemingly lost their ancestral macrosmatic abilities, biologically meaningful scents and some odours may still provide potential tools to modify human moods (Chen & Haviland-Jones, 1999) and/or behaviours. Schmeisser *et al.* (2013) have written extensively on scent applications for the military. Chemosensory cues of sickness can be detected by healthy individuals (humans) and used to limit contact with diseased individuals (Olsson *et al.*, 2014). In another recent study, human scents were used to match and identify individuals who were present or absent at a crime scene (Prada, Curran & Furton, 2010, 2014). Certainly, as technology improves, and our understanding of how to ascertain meaning within scents grows, the application of scents will transcend the disciplines that gave birth to this study (conservation and management).

VII. CONCLUSIONS

(1) The future of scent-based predator–prey studies is compelling. Much work is justified to understand and systematically document how semiochemicals provide reliable information to other animals under a range of contextual conditions (Fig. 3).

(2) We have shown how a number of catalysts from three complementary sub-disciplines (neurobiology, chemistry and ethology) can help us interpret past and future outcomes from predator–prey studies. Additionally, enhanced instrumentation and new protocols will allow us to fill in prominent knowledge gaps and understand

experimental inconsistencies that have emerged over the past few decades of research.

(3) Three complementary catalysts (neurobiological, chemical and context) interact to determine whether a scent is perceived as risky or attractive, and this has profound implications for designing effective scent-based tools.

(4) The neurobiological mechanism by which the scent is perceived may determine whether the scent is a limited noxious repellent or has potential for area-deterrence.

(5) Animals may respond to repeated scent exposure by either habituating or sensitizing; the contextual conditions that encourage sensitization are essential for the development of scent-based tools.

(6) The same predator scent can be perceived by prey as 'safe' or 'dangerous' depending on age, and conversely, can cause prey to be 'attracted' to risky cues through predator inspection.

(7) Advances in behavioural discrimination assays and new technology can help to account for and stabilize chemical fidelity in an uncertain environment (diet, degradation, transmission and placement).

(8) The number of avenues for scent application transcends disciplines, and warrants the highest level of inquiry into establishing more efficacious scents for applications from conservation to the military.

(9) Over the next decade, we must, once again, rethink what we know about prey responses to predator odours, as we revisit many of the same questions, armed with increased analytical and mobile instrumentation capabilities, as well as contemporary behavioural discrimination assays that are sufficiently sensitive to reveal a wide range of prey response behaviours (Zou *et al.*, 2015; Garvey, Glen & Pech, 2016). By appreciating the key contextual parameters that explain variation in responses, we will develop novel applications that were not previously possible.

VIII. ACKNOWLEDGEMENTS

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