Detecting the smell of disease and injury: scoping evolutionary and ecological importance.

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"By the sense of smell we can recognize the peculiar perspiration of many diseases, which has an important bearing on their identification."

Sushruta Samhita (an ancient Sanskrit text on medicine and surgery c. 6th Century BCE)

Puss, vomit, diarrhoea, blood, rank perspiration and halitosis – all terms that evoke repugnance amongst us due to their association with pain, disease and failing health; and all conditions linked with a primeval stench. Furthermore, although medical science has progressed from those ancient days of sniffing patients and tasting urine to keener diagnostics, it remains that certain illnesses can be identified by their distinctive aroma (after Penn & Potts, 1998):

- Arsenic poisoning - Garlicky body odour
- Cholera - Sweet-smelling faeces
- diabetic ketosis - a fruity aroma of decomposing apples
- diphtheria - sweet
- hyperaminoaciduria - dried malt or hops
- inability to metabolize methionine - boiled cabbage
- Phenylketonuria - Musty sweat and urine
- Pneumonia - Foul breath
- scrofula - stale beer
- typhoid fever - baked bread
- yellow fever - a butcher's shop

This phenomenon not only has medical applications, and in this chapter we explore how being able to sniff out a sick individual has profound survival advantages in the natural world, both in terms of

avoiding infectious conspecifics and being choosy ~~who~~ to mate with ~~to maximise the fitness of~~
~~resulting offspring~~, and also how to pick the easiest prey to pick on. But first, how does sickness
affect odour?

That's disgusting!

The subtlety with which animals can discriminate sickness in others is a rapidly developing field. For instance, lab rats avoid sick individual that emit airborne odorant ~~signals~~ when cytokines are released during an inflammatory ~~response~~ (reviewed extensively by Arakara et al. 2011). Moreover, it doesn't matter what triggers this inflammation, be it a response to bacteria or parasites, simple injury/contusion, or even from exposure to psychological stress challenges (e.g. foot-shock – resulting in stress-dependent neuro-inflammation in lab rats). And this response is broadly conserved across species, where pro-inflammatory cytokines (principally interleukin-1beta (IL-1 β) and tumour necrosis factor alpha (TNF- α) affect the central nervous system (CNS) and modify the physiology of the sick individual to yield aversive cytokine odour cues (Kent et al., 1992a; Aubert, 1999; Dantzer, 2001). Furthermore, pathogens and injury (indeed even just fear of injury / attack) cause the hypothalamus–pituitary–adrenal (HPA) axis and the sympathetic nervous system to be activated (Barnard et al., 1996; Morales-Montor et al., 2001; Corrêa-de-Santana et al., 2006), also cueing a stress response with the release of corticosteroids and epinephrine – the smell of fear.

Sickness odour cue are also present in excreted metabolites, in part arising due to changes in exocrine gland products, but also through the urine and faeces (e.g. Hurst & Beynon, 2004). Infection can induce changes in the intestinal flora through the composition of communal microbe – the so-called 'microflora hypothesis' (Howard, 1977; Schellinck et al., 1991) and/or either inhibit gastric emptying, or stimulate colonic propulsive motility causing diarrhoea (Brown and Schellinck, 1995) and the defecation of less digested excrement (Conte et al., 2006).

In terms of detection, ~~mammals~~ can smell specific gut products, such as ~~guanylin~~, with great sensitivity (Leinders-Zufall et al., 2007), as well as changes in volatile urinary and faecal acids (Zeng et al., 1996). Even humans respond in this way, despite our relatively low conscious reliance on olfaction. Sampling the body odour (BO) of trial participants injected with an endotoxin (lipopolysaccharide; LPS) to trigger an innate immune response revealed that after just four hours their BO became aversive compared to the BO of participants treated with placebo (Olsson et al. 2014). What's more, this chemosensory aversion was linked directly to levels of immune activation, where the authors posit changes in the chemical compounds comprising BO (such as E-3-methyl-2-hexenoic acid, 3-methyl-3-sulfanylhexasan-1-ol, and sulfanylalkanols) and effects on the fermentation

products of the microbiota living on the skin, along with endocrine activation, levels of corticosterone, and genetic interactions with MHC. Notably, however, work by Ackerl et al. (2002) found no correlation between salivary cortisol levels and axillary (arm-pit) odour evasiveness after a group of women watched various scary movies, favouring an exocrine/fermentation mechanism. Collectively, the evolved interpretation of such chemosensory cues allows potential social partners to realise they should “stay at a healthy distance”. For instance, Poirotte et al. (2017) have shown that mandrills use olfaction to avoid social interactions with parasitized conspecifics.

Of course, it's not just the infected conspecific that might smell 'off' due to bleeding wounds, suppurating injury, or immune responses, it is the actual pathogen itself. Just as invertebrate ectoparasites use chemosensory signals to find and choose hosts (Osterkamp et al. 1999), it seems highly plausible that hosts will have evolved the ability to sniff out areas where parasites lurk, although this remains remarkably untested. For instance, ticks produce arrestment (=assembly) pheromones and attraction–aggregation–attachment (AAA) pheromones to signal that a walking meal has arrived (Sonenshine, 2004); surely keen mammalian noses can pick up on these signals and cause that infested habitat to be avoided? Or maybe grazing that meadow is simply too attractive a prospect to the deer, irrespective of the burden? Certainly domestic dogs will avoid parks and meadows infested with ticks and drool extensively over any incumbent they encounter. Plausibly too, as an avenue of chemofactory research, ‘tick juice’ might well provide a natural repellent for deterring grazing vertebrate pests?

But the ‘natural selection’ of simply avoiding sickness is only part of the selective advantage equation, there are also inter-generational implications...and so next we examine mate choice and how olfactory cues enable weeding out disease prone genes, to enhance future population success.

Sexual Selection: The fragrance of rejection

In 1982, Hamilton and Zuk proposed their landmark hypothesis that animals should inspect a potential mate's urine and faecal odour to select for parasite-free / resistant status. Building on this, substantial work has shown that rodents (and other mammals) base part of their selection for mating partners on major histocompatibility complex (MHC) cell surface proteins that in part determine individual immuno-competency; an olfactory signal they readily detect from urine (e.g. Ehman & Scott, 2001). However, mice can take this further, and females will actively reject urine from males infected with the intestinal nematode, *Heligmosomoides polygyrus* (Ehman & Scott, 2001). Indeed several parasites have been shown to manipulate endocrine function odour and thus

the behaviour of their hosts (Kavaliers et al., 1997; Klein, 2000). For instance Kavaliers et al. (2003) report that female mice can discriminate between the odour of urine from males infested with the mouse louse *Polyplax serrata*, and uninfested males, and avoid the odours of lousy males.

Illustrative of our relatively poor sense of smell, our embarrassed attempts to stop our pet dogs from butt sniffing in the park juxtaposes canine sociality against a sense of human antisocial behaviour. With a sense of smell ten- to one hundred- thousand times better than our own, this olfactory handshake exposes the sex of the protagonists, their age, diet, mood and crucially their health. Now if this interaction is a prelude to mating then either party (although females are typically more discriminating) can reduce their risks of contacting disease by choosing with whom to mate wisely; moreover selecting for pathogen resistant genes will be advantageous to her offspring. Indeed, in some species (e.g., Tomás et al. 2007), having a healthy male partner (or female, in monogamous pair breeders; Kokko et al. 2003) may even be important to help provision progeny through neonatal development. There is a wealth of examples of this type of scent-based discrimination – although mostly from controlled lab experiments; for example, healthy male mice have been shown to avoid, and refuse to copulate with, females infected with the indirectly transmitted parasite, *Trichinella spiralis* (Edwards & Barnard, 1987). Similarly Moore (2002) showed that females infected with internal parasites such as, *T. crassiceps*, *Echinostoma revolutum*, and *E. caproni* exhibited decreased in fertility / fewer young.

Handicap

Penn and Potts (1998) elucidate the role of androgens in the immuno-competence handicap hypotheses in mate selection. Testosterone is immunosuppressive because it protects developing sperm that present novel antigens from autoimmune attack (Folstadt & Skarstein 1997; Sheldon & Verhulst, 1996). Consequently, males mounting an immunological response to pathogenic infections may be forced to reduce investment in sexual displays, including olfactory gland products (Sheldon & Verhulst, 1996). Interestingly, it is well-established that socially housed male rodents invest in dominance displays experience at a cost to immune capacity (Klein et al., 1997).

From the female recipients perspective, Kavaliers and Colwell (1993) found that the odour of parasitized male mice trigger a neuroendocrine analgesic mechanism, elevating –endorphins, enkephalins and non-opioid serotonergic levels to enhance pain tolerance. They hypothesized that this could be part of the general stress response, driven by anxiety if faced with an infected mating partner (Kavaliers and Colwell 1995a/b). Alternatively, this may be an odour-mediated preparatory defence mechanism; for instance opioids bind readily to receptors in the intestine, which could

alleviate some of the otherwise debilitating disease arising from coccidial diarrhoea (Stefano et al. 1996). Furthermore, opioids inhibit libido, and thus reduce lordosis, sexual receptivity and oestrous in rodents (Hammer et al., 1994).

Heterozygosity

We mentioned above olfactory cues relating to MHC, and lab mice and rats certainly prefer to mate with individuals expressing dissimilar MHC genes (e.g., Penn & Potts, 1998a/b). This can increase individual MHC-heterozygosity, to present a moving target to rapidly evolving pathogens (the 'Red Queen' hypothesis; Van Valen, 1977), or increase disease resistance by reducing inbreeding and promoting genomic heterozygosity (see Sin et al. 2014).

Stepping away from the lab, our own work led by YungWa (Simon) Sin on wild European badgers (*Meles meles*) has found that two MHC alleles confer qualitative and/or quantitative resistance to certain pathogens, while a further three alleles were associated with pathogen susceptibility (Sin et al. 2014), although MHC heterozygotes did not exhibit a lower pathogen burden or infection status. Indeed, contrary to the MHC-disassortative mating hypothesis (e.g. Juola and Dearborn 2011), which predicts general preference for MHC-dissimilar mates (generating offspring heterozygosity), we found that badgers were more likely to breed with MHC-similar mates (Sin et al. 2015). In several other species, MHC dissimilarity and heterozygosity have proven advantageous, however MHC diversity can cause a loss of T-cells, lowering targeted absolute immune capacity in favour of diversity (Lawlor et al. 1990), which also increases the risk of auto-immune diseases (Kaufman 1999; Puurtinen et al. 2005). Importantly, by far the most significant pathogen driving natural selection in this badger population, *Eimeria melis*, is a coccidian, perhaps presenting a singularly focused target for optimal immune profiles. As to whether badgers can detect one another's MHC genetics, and therefore gain information on immuno-competence, badgers have a unique subcaudal gland encoding individual specific parameters (Buesching et al. 2002a/b), and we have found that microbiota play a key role in metabolizing these primary gland products, according with the microflora hypothesis (Yamazaki et al. 1990; Lanyon et al. 2007). Plausibly MHC genes control odour in badgers indirectly by influencing the growth of microbiota commensal on subcaudal secretion, to yield characteristic scent profiles (Sin et al. 2012).

Sexually transmitted diseases (STDs)

One very obvious, yet under-investigated aspect of how smell could influence mate choice directly is

through diseases transmitted during coitus (Kokko et al. 2002). STDs can not only have direct effects on sexual performance that preclude mating attempts, but have subsequent cryptic implications, best avoided, such as trans-placental (vertical) transmission to offspring (either causing abortion or reduced lifetime fitness), or causing permanent damage to the reproductive organs of sexual partners, extinguishing future fitness.

In humans, various STDs are associated with vaginal discharges with distinctive 'fishy' odour and associated discomfort. Pathogenic agents causing odorous infection may be protozoan (e.g. Trichomoniasis), bacterial (e.g. Chlamydia, Syphilis / *Treponema pallidum*, Gonorrhoea / *Neisseria gonorrhoeae* – often linked to bloody discharge / vaginosis), or fungal (Candida / yeast). Additionally, physiological conditions such as cervical or vaginal cancer or rectovaginal fistulae cause distinctive smells, indicative of a compromised female reproductive system. These primary STDs can also lead to serious secondary conditions, also smelling nefarious, such as Mucopurulent cervicitis (MPC) and Pelvic Inflammatory Disease (PID). Viral STDs (e.g., genital herpes, HIV, Human papillomavirus - HPV) tend to cause less immediate odour, but are likely detectable through secondary olfactory changes. Similarly, in males, gonorrhoea, nongonococcal urethritis, syphilis and chlamydia cause smelly pus to drip from the penis, and can cause associated testicular swelling. Even conditions arising from poor hygiene can cause odour, such as the build up of smegma under the foreskin from accumulating un-cleaned oils and skin cells around the shaft of the penis. There can even be more subtle olfactory evidence of STDs; for example, a Russian study found that the smell of axillary sweat from men infected with gonorrhoea was distinguishable and aversively 'putrid' to women sniffers (Moshkin et al. 2012). Not least, conditions like gonorrhoea cause burning genital tract pain, causing behavioural changes and distinct loss of libido.

Outside of human reproductive biology, there is a much broader potential for contagion, with various venereal pathogens also present in urine, faeces and glandular secretions, exposing the broader population to more casual risks while traversing habitat – risks that may be avoided by detecting the scent of contaminants. Given work with disease detecting dogs showing canine ability to distinguish a variety of diseases in human patients, such as cervical / ovarian cancer from exhaled breath (Guerreo-Flores et al. 2017), or prostate cancer from urine (Cornu et al., 2011), the acute olfactory senses of wild-living animals must surely be able to detect and evade sexual infections?

Despite some seminal work on STDs in animals per se (e.g. Lockhart et al. 1996) there has been surprisingly little work in the wild (of which we are aware) relating olfactory cues to STDs, to mating avoidance. Nevertheless, game-theory predicts that if STD-risk matings can be avoided, then the rate of spread of STDs within a population would be curbed. In addition, any reduction in matings involving infected females would limit the vertical spread of infection to offspring. However, if

reproductive opportunities are scarce, or survival until the next mating season is unlikely, game theory would predict that animals (esp. males) might risk infection to maximise their reproductive output, thus mating indiscriminately (Terminal Investment Hypothesis) – a tactic modified by the pathogenicity of the STD, the risk of infection, recipient immune status, and the extent to which the disease impacts fecundity and subsequent offspring fitness. Relating to this, [Stevenson et al. \(2011\)](#) report that (in undergraduate males) arousal causes a distinct drop in sensitivity to sex-related disgust cues.

Developing some testable hypotheses from our own focal wild badger model species (reviewed in [Macdonald et al. 2015](#)), key questions would be to:

- 1) To determine the population-level prevalence of a wide variety of bacterial, viral, and protozoan STD pathogens, using a large-scale screening approach to also detect asymptomatic infections and characterise modes of transmission;
- 2) To investigate individual consequences of STDs on life-time reproductive success (LRS) as determined by genetic pedigree analyses;
- 3) To investigate secondary fitness consequences of STDs in terms of odour changes in olfactory reproductive advertisement signals as determined through GCMS analyses;

4) To test if individuals modify their reproductive behaviour, mate choice and choice of social partners according to these STD-related odour cues, using large-scale scent-provisioning experiments;

5) To develop a game-theoretical model exploring the viability of odour-based mate choice as an ESS.

For instance, top STD candidate species for investigation in badgers include *Neospora caninum* (causing abortion in other species; [Bartley et al., 2013](#)) and *Toxoplasma gondii* (causing still births and abortion in other species; [Anwar et al., 2006](#)). Our own pilot screening has also detected (Dugdale & CDB unpubl) *Chlamydia abortus* infection in genital discharge (causing abortion in American mink, *Neovison vison*, and pine marten, *Martes martes* [ref.](#)). We have found genital tract Mustelid herpes virus (MusGHV-1; using DNA sequencing; [Kent et al., 2017](#)) in 54 of 98 swabs collected from 71 female badgers, which sits against 354 of 361 of blood samples from 218 mixed sex badgers being MHV positive by PCR, suggesting strong horizontal but also likely vertical transmission. Furthermore, using 16S sequencing, we found *Staphylococcus intermedius* in badger subcaudal gland secretion (known to cause pyometra, vaginitis and mastitis in American mink [Bartley et al., 2013](#)), *E. coli* (known to cause vaginitis and pyometra in ferrets *Mustela furo* [ref.](#)) as well as *Enterococcus faecalis* (known to cause substantial mucosal epithelial ulceration of the uterus in Siberian polecats, *Mustela eversmanni* [ref.](#)).

Implications for ecology and survival: Then fragrance of fragility

The way in which carnivores use scent (urine, faeces, glandular secretions) to mark their territories and communicate resource holding potential has grown as a paradigm since the early work of [Brown and Macdonald \(1985\)](#), and [Morris Gosling \(1986\)](#), *inter alia*.

More recently, it has become apparent that prey species 'eaves drop' ([Hughes et al. 2010](#)) on the conversations going on between their predators to try to avoid risky encounters in both time and space ([see Chapter xxx, this volume](#)), where the smell of predator odour broadly induces fear ([Takahashi et al., 2005](#)).

Of course, there is a very fundamental understanding that many predators (from mammals to snakes to sharks) sniff out their prey – indeed this is the very basis for how dogs became 'man's best friend' in the hunt, scenting quarry and following blood trails ([Budgett & Edwards 1933](#)); a union now harnessed in a variety of 'scent detection dog' applications, from narcotics to explosives ([Browne et al 2006](#); [Byrd et al. 1996](#)). Work led by Nelika Hughes has highlighted that wild living rodents are well aware that their intraspecific scent marks are being snooped on by predators, with

domestic mice trading off risky scent receiving behaviour in the presence of cat urine (Hughes et al. 2009), in-so-far as the signaller has by definition left, leaving the sniffer standing by the 'come eat me' cue. Performing similar work in a more complex system involving weasel predation on two competing Finland voles, sibling voles *Microtus rossiameridionalis* and field voles *M. agresti*, Hughes et al. (2010) showed that multi-species assemblages are clearly sensitive to the potential for increased predation risk, trading off how conspicuous they can afford their scent signals to be. A similar scenario plays out in the marine environment; Lönnstedt et al. (2012) found that well-fed fish conditioned with visual, chemical or a combination of predator cues survived eight times better over the first 48 h on reefs than those with no experience of benthic predator cues.

But in the context of our chapter here, let's take this a step further, if a shrew is out hunting for weevils, will it deliberately seek out 'the lesser of two weevils' (Andersen & Folk, 1993). If a tiger hunts sambar deer (O'Brien et al. 2003) will it deliberately prioritise stalking weaker, diseased individuals? Importantly, from our perspective, using all of those cues of disease we described as leaking out, above, in the context of intraspecific social avoidance and mate selection? According to game theory, it should, and limited evidence does point to those individual prey caught by predators tending to be those in poorer physical or behavioural condition (Gosling & Petrie 1981).

Injured individuals across taxa produce alarm odours ('Schreckstoff') linked to freezing or flight, which can even elicit mobbing by conspecifics (Muller-Schwarze, 1980; Pfeiffer, 1962). While it is well established that wolves follow blood trails (Gable et al. 2016), and sharks use olfaction to detect blood in the water (Tester, 1963), there is a profound lack of studies examining if predators scent prey quality, to pre-inform themselves on how to hasten the demise of the weakest. In evolutionary terms the advantages of securing the easiest possible meal should be a 'no brainer' and under strong selection pressure, fundamental to the very foundation of Darwinian fitness. Interestingly though it appears knowledge of prey scent is not innate and must be learnt (Apfelbach, 1973).

In a rare but important examination of this topic Robert Cocke and Del Thiessen (1986) describe how predators can use these alarm odours to localise prey and to assess their defensive / escape capabilities; be these least weasels (*Mustela nivalis*) snooping on the quality of deer mice (*Peromyscus bairoli*) (Cushing, 1984), or garter snakes (*Thamnophis sirtalis*) scenting out which individual prey in would best pay to hunt (Burghardt, 1970). Cocke and Thiessen (1986) demonstrated experimentally that captive Mongolian gerbils (*Meriones unguiculatus*) become anxious upon smelling the 'blood odour' collected from an individual subjected to a stressor – indicating that stressed or injured rodents avoid each other. Why? Well, in a second experimental phase they found that foxes (*Vulpes vulpes*), ferrets (*Mustela putorius furo*), and domestic cats (*Felis*

domestica) all actively sought out this blood odour from stressed gerbils. The precise semiochemical physiology involved in producing this blood odour was, however, not established; however Marks (1988) speculates that blood-injury phobia in people evolved so that we would stay away from injured peers likely to draw the interest of predators.

More recently, Barreto et al. (2013) found a similar story for Nile tilapia (*Oreochromis niloticus*), which display an anti-predator response to chemical cues present in the blood of conspecifics (the first report of an alarm response induced by blood-borne chemical cues in fish). In this instance, it seemed that chemical cues were produced by epidermal 'club' cells, and elicited an alarm reaction in fellow fish.

Beyond scenting blood – or unspecified cue sources within blood, the extent to which predators eavesdrop on prey infirmity via the manifest other cues they produce, including their faeces and urine, is yet to be fully realised. Hudson et al (1992) provide some intriguing evidence from the arena of game bird rearing, noting that those grouse killed by predators prior to the shoot had higher caecal nematode (*Trichostrongylus tenuis*) counts (from post-mortem) than grouse ultimately shot during the autumn. Furthermore, the proportion of grouse with high levels of parasite infection increased with the intensity of predator control as measured indirectly through gamekeeper effort, suggesting that predators selectively preyed on heavily infected grouse. Fascinatingly, this study used an oral anthelmintic to experimentally by reduce grouse nematode burdens, but it proved much harder for specially trained dogs to locate the nests of treated birds, implying that parasite-free grouse smelled less. No olfactory mechanism was investigated here, but it is grist to our mill. Aside from these highly instructive exceptions, research on scenting disease and infirmity in prey is scant within an otherwise multi-faceted olfactory predator-prey paradigm, despite our fairly exhaustive literature searches.. We thus encourage studies to question further the extent to which predators make decisions on prey quality (resistance to predation) based on leaky scent cues.

There are, inevitably, also caveats to this scenting frailty hypothesis – again testable ones: choosing to stalk weak injured or diseased prey, as evidenced from their olfactory cues, ceases to make such good evolutionary sense, if that prey carries a communicable parasite. In such cases the discerning predator may choose to steer clear of that particular dinner (provided it is not already infected, of course). If scent cues, say, the *Toxoplasma gondii* infection status of a rat to a Lynx – and *T. gondii* infection does smell, in-so-far that uninfected female rats, oddly, prefer the smell of infected males (a further marvel of evolved olfactory manipulation through heightening testosterone odour in urine; Vyas, 2013) – does that Lynx avoid choosing said rat? Of course, in the ultimate evolutionary Red Queen game (Van Valen, 1977) this scenario is compromised by the fact that *Toxoplasma* blocks

the innate aversion rodents typically have to cat urine, instead producing an attraction to pheromones that increase the likelihood of being predated (Vyas et al. 2007).

As with simple eavesdropping, this more sophisticated 'qualitative' eavesdropping on scent cues might also work both ways: Take two neighbouring territorial bobcats, one is old, infirm and presenting anorexia, leukopaenia, neutropaenia, lymphopaenia, and nonregenerative anaemi – all indicative of Feline Leukaemia Virus (FeLV; Sleeman et al. 2001), whereas the other is young, fit and healthy. Any snowshoe hare deciding where to live would logically decide to take its chances living alongside the former, all else being equal: Another testable hypothesis.

Conclusion

As Dietland Muller-Schwarze predicted in his preface to the CSiV 13 book (year), a lot of the progress in the field of semio-chemistry have indeed arisen through technological advances, and new avenues such as proteomics (see chapter xx, this volume). Nevertheless, as we scope out here, there remains a wealth of evolutionary and ecological applications these techniques can be applied to, where this sensory modality, so long too cryptic for us to properly comprehend, is fundamental to understanding species natural history. Now that it is well-established that vertebrates use scent cues to inform themselves on the disease, injury and immune status of conspecifics in the context of social contact networks and mate choice, long-term datasets may reveal whether there is evidence that olfaction is a functional mechanism contributing to sexual selection. Furthermore, given the arms-race between eavesdropping predators and prey on how conspicuous scent marks might be deployed and spied upon, a vital component of understanding how predators select which individual prey to hunt (or not) may well be tied to semiochemical cues.

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References

Ackerrl, K., Atzmueller, M., & Grammer, K. (2002). The scent of fear. *Neuroendocrinology Letters*, 23(2), 79-84.

Andersen, D. C., & Folk, M. L. (1993). Blarina brevicauda and Peromyscus leucopus reduce overwinter survivorship of acorn weevils in an Indiana hardwood forest. *Journal of Mammalogy*, 74(3), 656-664.

Anwar, A., Knaggs, J., Service, K. M., McLaren, G. W., Riordan, P., Newman, C., ... & Macdonald, D. W. (2006). Antibodies to Toxoplasma gondii in Eurasian badgers. *Journal of wildlife diseases*, 42(1), 179-181.

Apfelbach, R. (1973). Olfactory Sign Stimulus for Prey Selection in Polecats (Putorius putorius L.) 1. *Zeitschrift für Tierpsychologie*, 33(3-4), 270-273.

Arakawa, H., Cruz, S., & Deak, T. (2011). From models to mechanisms: odorant communication as a key determinant of social behavior in rodents during illness-associated states. *Neuroscience & Biobehavioral Reviews*, 35(9), 1916-1928.

Aubert, A. (1999). Sickness and behaviour in animals: a motivational perspective. *Neuroscience & Biobehavioral Reviews*, 23(7), 1029-1036.

Barreto, R. E., Miyai, C. A., Sanches, F. H. C., Giaquinto, P. C., Delicio, H. C., & Volpato, G. L. (2013). Blood cues induce antipredator behavior in Nile tilapia conspecifics. *PLoS One*, 8(1), e54642.

Bartley, P. M., Wright, S. E., Zimmer, I. A., Roy, S., Kitchener, A. C., Meredith, A., ... & Katzer, F. (2013). Detection of Neospora caninum in wild carnivores in Great Britain. *Veterinary parasitology*, 192(1-3), 279-283.

Bernard, J. F., Bester, H., & Besson, J. M. (1996). Involvement of the spino-parabrachio-amygdaloid and-hypothalamic pathways in the autonomic and affective emotional aspects of pain. In *Progress in brain research* (Vol. 107, pp. 243-255). Elsevier.

Bird, R. C. (1996). An examination of the training and reliability of the narcotics detection dog. *Ky. LJ*, 85, 405.

Brown and Macdonald 1985

Brown, R.E., Schellinck, H.M., 1995. Effects of selective depletion of gut bacteria on theodoursof individuality in rats. In: Apfelbach, R., Muller-Schwarze, D., Reutter, K., Weiler, E. (Eds.), *Chemical Signals in Vertebrates*, VII. Pergamon Press, New York, pp. 267–271

Browne, C., Stafford, K., & Fordham, R. (2006). The use of scent-detection dogs. *Irish Veterinary Journal*, 59(2), 97.

Budgett, H. M., & Edwards, L. (1933). *Hunting by scent*. Charles Scribner's Sons.

Buesching, C. D., Waterhouse, J. S., & Macdonald, D. W. (2002a). Gas-chromatographic analyses of the subcaudal gland secretion of the European badger (*Meles meles*) part I: chemical differences related to individual parameters. *Journal of Chemical Ecology*, 28(1), 41-56.

Buesching, C. D., Waterhouse, J. S., & Macdonald, D. W. (2002b). Gas-chromatographic analyses of the subcaudal gland secretion of the European badger (*Meles meles*) Part II: time-related variation in the individual-specific composition. *Journal of Chemical Ecology*, 28(1), 57-69.

Cocke, R., & Del Thiessen, D. (1986). Chemocommunication among prey and predator species. *Animal Learning & Behavior*, 14(1), 90-92.

Conte, M. P., Schippa, S., Zamboni, I., Penta, M., Chiarini, F., Seganti, L., ... & Cucchiara, S. (2006). Gut-associated bacterial microbiota in paediatric patients with inflammatory bowel disease. *Gut*, 55(12), 1760-1767.

Cornu, J. N., Cancel-Tassin, G., Ondet, V., Girardet, C., & Cussenot, O. (2011). Olfactory detection of prostate cancer by dogs sniffing urine: a step forward in early diagnosis. *European urology*, 59(2), 197-201.

Corrêa-de-Santana, E., Paez-Pereda, M., Theodoropoulou, M., Nihei, O. K., Gruebler, Y., Bozza, M., ... & Stalla, G. K. (2006). Hypothalamus–pituitary–adrenal axis during *Trypanosoma cruzi* acute infection in mice. *Journal of Neuroimmunology*, 173(1-2), 12-22.

Cushing, B. S. (1984). A selective preference by least weasels for oestrous versus dioestrous urine of prairie deer mice. *Animal Behaviour*, 32(4), 1263-1265.

Dantzer, R. (2001). Cytokine-induced sickness behavior: mechanisms and implications. *Annals of the New York Academy of Sciences*, 933(1), 222-234.

Edwards, J. C., & Barnard, C. J. (1987). The effects of *Trichinella* infection on intersexual interactions between mice. *Animal Behaviour*, 35(2), 533-540.

Ehman, K. D., & Scott, M. E. (2001). Urinary odour preferences of MHC congenic female mice, *Mus domesticus*: implications for kin recognition and detection of parasitized males. *Animal Behaviour*, 62(4), 781-789.

Folstad, I., & Skarstein, P. (1997). Is male germ line control creating avenues for female choice?. *Behavioral Ecology*, 8(1), 109-112.

Gable, T. D., Windels, S. K., Bruggink, J. G., & Homkes, A. T. (2016). Where and how wolves (*Canis lupus*) kill beavers (*Castor canadensis*). *PloS one*, 11(12), e0165537.

- Gosling, L. M. (1986). Economic consequences of scent marking in mammalian territoriality. In *Chemical signals in vertebrates 4* (pp. 385-395). Springer, Boston, MA.
- GOSLING, L.M., & PETRIE, M. (1981). The economics of social organization. In: C.R. Townsend & P. Calow (Eds.), *Physiological ecology* (pp. 315-345). Oxford: Blackwell Scientific Publications.
- Guerrero-Flores, H., Apresa-García, T., Garay-Villar, Ó., Sánchez-Pérez, A., Flores-Villegas, D., Bandera-Calderón, A., ... & Mata, O. (2017). A non-invasive tool for detecting cervical cancer odor by trained scent dogs. *BMC cancer*, 17(1), 79.
- Hamilton, W. D., & Zuk, M. (1982). Heritable true fitness and bright birds: a role for parasites?. *Science*, 218(4570), 384-387.
- Hammer Jr, R. P., Zhou, L., & Cheung, S. (1994). Gonadal steroid hormones and hypothalamic opioid circuitry. *Hormones and behavior*, 28(4), 431-437.
- Hudson, P. J., Dobson, A. P., & Newborn, D. (1992). Do parasites make prey vulnerable to predation? Red grouse and parasites. *Journal of animal ecology*, 681-692.
- Hughes, N. K., Kelley, J. L., & Banks, P. B. (2009). Receiving behaviour is sensitive to risks from eavesdropping predators. *Oecologia*, 160(3), 609-617.
- Hughes, N. K., Korpimäki, E., & Banks, P. B. (2010). The predation risks of interspecific eavesdropping: weasel-vole interactions. *Oikos*, 119(7), 1210-1216.
- Hughes, N. K., Korpimäki, E., & Banks, P. B. (2010). The predation risks of interspecific eavesdropping: weasel-vole interactions. *Oikos*, 119(7), 1210-1216.
- Hurst, J. L., & Beynon, R. J. (2004). Scent wars: the chemobiology of competitive signalling in mice. *Bioessays*, 26(12), 1288-1298.
- JC, Howard (1977). H-2 and mating preferences. *Nature*, 266(5601), 406-408.
- Juola, F. A., & Dearborn, D. C. (2011). Sequence-based evidence for major histocompatibility complex-disassortative mating in a colonial seabird. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20110562.
- Kaufman, J. (1999). Co-evolving genes in MHC haplotypes: the "rule" for nonmammalian vertebrates?. *Immunogenetics*, 50(3-4), 228-236.
- Kavaliers, M., & Colwell, D. D. (1993). Aversive responses of female mice to the odors of parasitized males: neuromodulatory mechanisms and implications for mate choice. *Ethology*, 95(3), 202-212.

- Kavaliers, M., & Colwell, D. D. (1995a). Discrimination by female mice between the odours of parasitized and non-parasitized males. *Proc. R. Soc. Lond. B*, 261(1360), 31-35.
- Kavaliers, M., & Colwell, D. D. (1995b). Odours of parasitized males induce aversive responses in female mice. *Animal Behaviour*, 50(5), 1161-1169.
- Kavaliers, M., Colwell, D. D., Ossenkopp, K. P., & Perrot-Sinal, T. S. (1997). Altered responses to female odors in parasitized male mice: neuromodulatory mechanisms and relations to female choice. *Behavioral Ecology and Sociobiology*, 40(6), 373-384.
- Kavaliers, M., Fudge, M. A., Colwell, D. D., & Choleris, E. (2003). Aversive and avoidance responses of female mice to the odors of males infected with an ectoparasite and the effects of prior familiarity. *Behavioral Ecology and Sociobiology*, 54(5), 423-430.
- Kent, A., Ehlers, B., Mendum, T., Newman, C., Macdonald, D. W., Chambers, M., & Buesching, C. D. (2018). Genital Tract Screening Finds Widespread Infection with Mustelid Gammaherpesvirus 1 in the European Badger (*Meles meles*). *Journal of wildlife diseases*, 54(1), 133-137.
- Kent, S., Bluthé, R. M., Dantzer, R., Hardwick, A. J., Kelley, K. W., Rothwell, N. J., & Vannice, J. L. (1992). Different receptor mechanisms mediate the pyrogenic and behavioral effects of interleukin 1. *Proceedings of the National Academy of Sciences*, 89(19), 9117-9120.
- Klein, S. L. (2000). Hormones and mating system affect sex and species differences in immune function among vertebrates. *Behavioural processes*, 51(1-3), 149-166.
- Klein, S. L., Hairston, J. E., DeVries, A. C., & Nelson, R. J. (1997). Social environment and steroid hormones affect species and sex differences in immune function among voles. *Hormones and Behavior*, 32(1), 30-39.
- Kokko, H., Brooks, R., Jennions, M. D., & Morley, J. (2003). The evolution of mate choice and mating biases. *Proceedings of the Royal Society of London B: Biological Sciences*, 270(1515), 653-664.
- Kokko, H., Ranta, E., Ruxton, G., & Lundberg, P. (2002). Sexually transmitted disease and the evolution of mating systems. *Evolution*, 56(6), 1091-1100.
- Lanyon, C. V., Rushton, S. P., O'donnell, A. G., Goodfellow, M., Ward, A. C., Petrie, M., ... & Penn, D. J. (2007). Murine scent mark microbial communities are genetically determined. *FEMS microbiology ecology*, 59(3), 576-583.
- Lawlor, D. A., Zemmour, J., Ennis, P. D., & Parham, P. (1990). Evolution of class-I MHC genes and proteins: from natural selection to thymic selection. *Annual review of immunology*, 8(1), 23-63.

- Leinders-Zufall, T., Cockerham, R. E., Michalakis, S., Biel, M., Garbers, D. L., Reed, R. R., ... & Munger, S. D. (2007). Contribution of the receptor guanylyl cyclase GC-D to chemosensory function in the olfactory epithelium. *Proceedings of the National Academy of Sciences*, *104*(36), 14507-14512.
- Lockhart, A. B., Thrall, P. H., & Antonovics, J. (1996). Sexually transmitted diseases in animals: ecological and evolutionary implications. *Biological Reviews*, *71*(3), 415-471.
- Lönnerstedt, O. M., McCormick, M. I., Meekan, M. G., Ferrari, M. C., & Chivers, D. P. (2012). Learn and live: predator experience and feeding history determines prey behaviour and survival. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20112516.
- Macdonald, D.W., Newman, C. & Buesching, C.D. (2015) Badgers in the rural landscape – Conservation paragon or farming pariah: Lessons from the he Wytham Badger Project. Pp65-95 . In: Wildlife Conservation on Farmland. Volume 2: Conflict in the Countryside (Eds DW Macdonald & RE Feber).
- Marks, I. (1988). Blood-injury phobia: a review. *The American journal of psychiatry*, *145*(10), 1207.
- Moore, J., 2002. Parasites and the Behavior of Animals. Oxford Univ.Press, New York.
- Morales-Montor, J., Mohamed, F., Ghaleb, A. M., Baig, S., Hallal-Calleros, C., & Damian, R. T. (2001). In vitro effects of hypothalamic–pituitary–adrenal axis (HPA) hormones on *Schistosoma mansoni*. *Journal of Parasitology*, *87*(5), 1132-1139.
- Moshkin, M., Litvinova, N., Litvinova, E. A., Bedareva, A., Lutsyuk, A., & Gerlinskaya, L. (2012). Scent recognition of infected status in humans. *The journal of sexual medicine*, *9*(12), 3211-3218.
- Müller-Schwarze, D. (1980). Chemical signals in alarm behavior of deer. In *Chemical Signals* (pp. 39-51). Springer, Boston, MA.
- O'Brien, T. G., Kinnaird, M. F., & Wibisono, H. T. (2003). Crouching tigers, hidden prey: Sumatran tiger and prey populations in a tropical forest landscape. *Animal Conservation*, *6*(2), 131-139.
- Olsson, M. J., Lundström, J. N., Kimball, B. A., Gordon, A. R., Karshikoff, B., Hosseini, N., ... & Axelsson, J. (2014). The scent of disease: human body odor contains an early chemosensory cue of sickness. *Psychological science*, *25*(3), 817-823.
- Osterkamp, J., Wahl, U., Schmalfuss, G., & Haas, W. (1999). Host-odour recognition in two tick species is coded in a blend of vertebrate volatiles. *Journal of Comparative Physiology A*, *185*(1), 59-67.
- Penn, D., & Potts, W. K. (1998). Chemical signals and parasite-mediated sexual selection. *Trends in Ecology & Evolution*, *13*(10), 391-396.

Penn, D., & Potts, W. K. (1998b). Untrained mice discriminate MHC-determined odors. *Physiology & Behavior*, 64(3), 235-243.

Pfeiffer, W. (1962). The fright reaction of fish. *Biological Reviews*, 37(4), 495-511.

Poirotte, C., Massol, F., Herbert, A., Willaume, E., Bomo, P. M., Kappeler, P. M., & Charpentier, M. J. (2017). Mandrills use olfaction to socially avoid parasitized conspecifics. *Science Advances*, 3(4), e1601721.

Puurtinen, M., Ketola, T., & Kotiaho, J. S. (2005). Genetic compatibility and sexual selection. *Trends in Ecology & Evolution*, 20(4), 157-158.

Schellinck, H. M., Brown, R. E., & Slotnick, B. M. (1991). Training rats to discriminate between the odors of individual conspecifics. *Animal Learning & Behavior*, 19(3), 223-233.

Sheldon, B. C., & Verhulst, S. (1996). Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in ecology & evolution*, 11(8), 317-321.

Sin, Y. W., Annavi, G., Dugdale, H. L., Newman, C., Burke, T., & Macdonald, D. W. (2014). Pathogen burden, co-infection and major histocompatibility complex variability in the European badger (*Meles meles*). *Molecular ecology*, 23(20), 5072-5088.

Sin, Y. W., Dugdale, H. L., Newman, C., Macdonald, D. W., & Burke, T. (2012). MHC class II genes in the European badger (*Meles meles*): characterization, patterns of variation, and transcription analysis. *Immunogenetics*, 64(4), 313-327.

Sleeman, J. M., Keane, J. M., Johnson, J. S., Brown, R. J., & Woude, S. V. (2001). Feline leukemia virus in a captive bobcat. *Journal of Wildlife Diseases*, 37(1), 194-200.

Smell Disease_Refs

Sonenshine, D. E. (2004). Pheromones and other semiochemicals of ticks and their use in tick control. *Parasitology*, 129(S1), S405-S425.

Stefano, G. B., Scharrer, B., Smith, E. M., Hughes Jr, T. K., Magazine, H. I., Bilfinger, T. V., ... & Makman, M. H. (1996). Opioid and opiate immunoregulatory processes. *Critical Reviews™ in Immunology*, 16(2).

Stevenson, R. J., Case, T. I., & Oaten, M. J. (2011). Effect of self-reported sexual arousal on responses to sex-related and non-sex-related disgust cues. *Archives of sexual behavior*, 40(1), 79-85.

Takahashi, L. K., Nakashima, B. R., Hong, H., & Watanabe, K. (2005). The smell of danger: a behavioral and neural analysis of predator odor-induced fear. *Neuroscience & Biobehavioral Reviews*, 29(8), 1157-1167.

- Tester, A. L. (1963). The role of olfaction in shark predation. *Pacific Science*, 17: 148-170.
- Tomás, G., Merino, S., Moreno, J., Morales, J., & Martinez-De La Puente, J. (2007). Impact of blood parasites on immunoglobulin level and parental effort: a medication field experiment on a wild passerine. *Functional Ecology*, 21(1), 125-133.
- Urghardt, G. M. (1970). Chemical perception in reptiles. In J. W. Johnson, D. G. Moulton, & A. Turk (Eds.), *Communication by chemical signals* (pp. 241-308). New York: Appleton-Century-Crofts.
- Van Valen, L. (1977). The red queen. *The American Naturalist*, 111(980), 809-810.
- Vyas, A. (2013). Parasite-augmented mate choice and reduction in innate fear in rats infected by *Toxoplasma gondii*. *Journal of Experimental Biology*, 216(1), 120-126.
- Vyas, A., Kim, S. K., Giacomini, N., Boothroyd, J. C., & Sapolsky, R. M. (2007). Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. *Proceedings of the National Academy of Sciences*, 104(15), 6442-6447.
- Yamazaki, K., Beauchamp, G. K., Imai, Y., Bard, J., Phelan, S. P., Thomas, L., & Boyse, E. A. (1990). Odor types determined by the major histocompatibility complex in germfree mice. *Proceedings of the National Academy of Sciences*, 87(21), 8413-8416.
- Zeng, X. N., Leyden, J. J., Spielman, A. I., & Preti, G. (1996). Analysis of characteristic human female axillary odors: qualitative comparison to males. *Journal of chemical ecology*, 22(2), 237-257.