Scent-matching of olfactory samples via proxy by domestic dogs *Canis lupus familiaris*

Shannon McKay

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science

Johannesburg

May 2014

Declaration

I declare that this dissertation is my own unaided work. It is being submitted for the degree Master of Science to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination to any other university.

_____

Shannon McKay

21 May 2014

Abstract

The well known scenting ability of domestic dogs has been frequently harnessed by using olfactory sampling of items imperceptible to a human sense of smell. Two main applications exist, which are a) detection of a particular scent, and b) scent-matching of a reference scent to a target scent in the presence of scented decoys. Studies on scent-matching have been limited to humans, where the scent vector is a proxy onto which the scent donor has transferred scent, and two non-human studies, in which the scat of the target species was used as the scent vector. All of these studies of scent-matching have been limited to individual scent donors of the same species. The aim of my study was to explore whether dogs can be trained to match a scent-sample via proxy in the presence of scented decoy proxies from other species, i.e. whether dogs are capable of scent-matching via proxy among several scent donor species. The experimental apparatus was a unique circular device with modifications based on previous research and my personal experience. Proxies were scented by a total of 15 species, by transferring scent onto the proxy either via scent donor body contact or scent donor by-product contact (i.e. scat, dung or shed skin of the scent donor). Training progressed in nine stages over a period of 13 months with three training groups. Fifteen subjects were recruited, with 12 completing the training process. All subjects which completed training underwent 80 runs (i.e. attempts to match a reference proxy to a target proxy). All subjects were able to correctly match a scented target proxy in the presence of five scented decoy proxies from other species at levels significantly greater than chance with an overall success rate of 66%. No early training predictors of eventual success were observed. Sex, breed and training venue did not influence success rates. Age of the subject did influence success rates, with the older and younger subjects having a significantly greater match success rate than subjects in the middle-age range. Proxy scenting method (skin contact or by-product contact) did not influence success rates and species of scent donor was also not found to be associated with success rates. My study demonstrated the use of an inexpensive and simple tool which could provide researchers with the means to explore general olfaction theories as well as more specific questions relating to the scenting ability of dogs for academic, commercial or civil purposes.

Acknowledgements

Thanks are expressed to Prof. Neville Pillay, Dr. Megan Jones, Dr. Megan Parker and Prof. Stuart Sym for their input and guidance and to Ashadee Kaye-Miller, Yolanda Beukes, Dr. Leon Koekemoer and Prof. Graham Alexander for their assistance. This study would not have been possible without the dog handlers and the dogs. Immense gratitude is thus extended to Ashadee Kaye-Miller (Zuri), Sally Tarlton (Zac), Marietjie Coetzee (Nemo), Ruth Bots (Maggie), Louise Kaye-Eddie (Foof), Maria Branco (Molly and Paco), Belinda Thomas (Scout), Vanessa Koekemoer (Emily), Helga Henckel (Hopi), Mimi Neumann (Chaska), Adrienne Hawkins (Oscar and Charles) and Jenny Juzi (Bobby and Victor) for their considerable contribution.

Finally, I would like to thank the University of the Witwatersrand, without whose support this study could not have been undertaken.

Animal Ethics clearance number: AESC 2011/26/2A.

Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction

1.1 Canine Scenting Ability

The extraordinary olfactory acuity of domestic dogs *Canis lupus familiaris* is well known among researchers (Szetei *et al*, 2003; Browne *et al*, 2006; Richards *et al*, 2008; Walczak *et al*, 2009). It is thought that this ability has been derived from the shared wolf *Canis lupus* ancestor, which used this heightened sense in order to locate food, avoid danger and recognise kin. In studies which compared the human sense of smell to that of the domestic dog, dogs are on average 100 to 1 000 times better than humans (Szetei *et al*, 2003) at detecting the presence of non-biological odours (e.g. explosives) and biological scents (e.g. animal by-products such as scat). Humans cannot match the sensitivity and accuracy of canine olfaction and to date there is no mechanical or electronic device capable of matching the precision of the canine olfactory system (Brisbin *et al*, 1991; Sommerville *et al*, 1993; Settle *et al*, 1994).

In general, dogs are known to perform well at operant conditioning tasks (Meyer & Ladewig, 2008; Jezierski *et al*, 2010; Demant *et al*, 2011) where a response from the dog can be elicited after a verbal or hand signal cue is given to the dog by the handler and it responds with the desired response in the anticipation of a reward. This ease of operant conditioning in dogs allows for their olfactory skills to be used by humans to detect and locate the source of odours and scents, which are undetectable to humans. A review by Browne *et al* (2006) lists the olfaction training applications of dogs to include: odours (explosives, land mines, arson accelerants, hazardous chemicals and narcotics), human scents (crime scene identification, missing people, disaster victims, cadaver recovery, cancer detection and seizure alerts) and non-human scents (invasive species, crop-damaging insects, termites, internal parasites, oestrus in production animals, carcasses of birds at wind farms and scats of endangered species). The canine nose is thus a very useful tool across many research and applied fields with a broad scope of utility.

1.2 Scent Training Applications

With appropriate training, dogs can either detect the source of a target scent (Smith *et al*, 2003; Fukuhara *et al*, 2010) or follow a “match to sample” protocol (Settle *et al*, 1994; Schoon, 1996; Schoon, 2005, Jezierski *et al*, 2010). For the purposes of clarity in this report, I shall refer to dogs trained to find a particular target scent as detection dogs and dogs trained

to the "match to sample" protocol as scent-matching dogs (referred to collectively as scent dogs).

Detection dogs are generally trained to establish a long-term olfactory memory (Smith *et al*, 2003; Fukuhara *et al*, 2010) and to find the source of this target scent using the actual scent-producing article (Figure 1). For example, a scat detection dog could be trained to locate a cheetah *Acinonyx jubatus* scat by operant conditioning to a cheetah scat. Once proficient, the detection dog would be used to find cheetah scats in nature. In biological applications, this tool can assist researchers in establishing whether a species, such a cheetah in the example above, is present in an area.

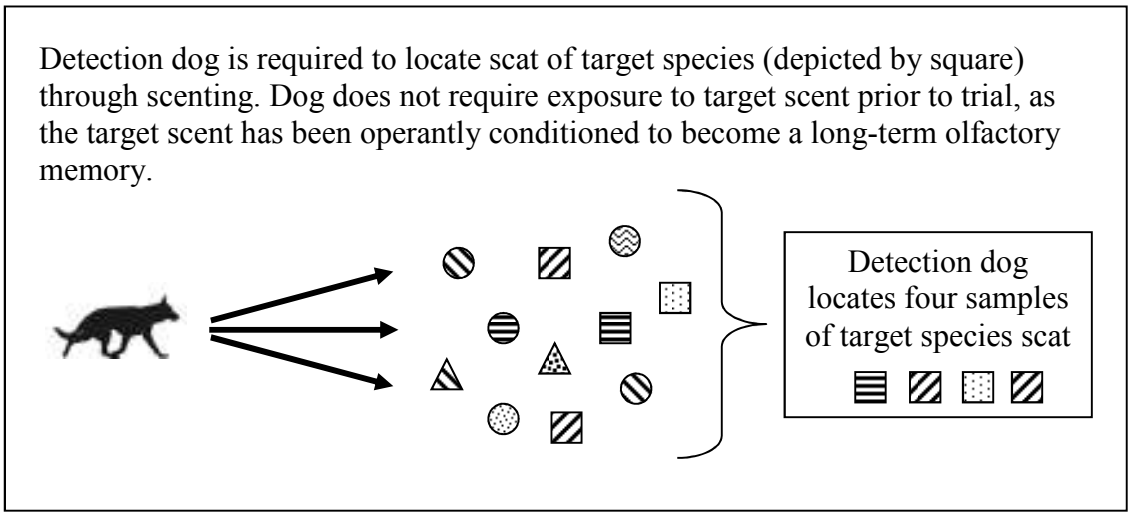

Figure 1. Graphic illustration of the detection dog concept in a hypothetical experiment to establish species presence in a survey area by detecting scats of a target species on which the detection dog has been operantly conditioned.

In contrast to detection dogs, a scent-matching dog is taught to match a scent presented to the dog with one of a number of options available. To the best of my knowledge in the literature, this has only ever been studied at an individual level, i.e. the dog has been presented with a number of olfactory samples of individuals of the same species and the dog has been required to match individuals within that sample of that species (Figure 2). Scent-matching by dogs of individuals within a species has been studied in three scent-donor species – Amur tigers *Panthera tigris altaica* (Kerley & Salkina, 2007), maned wolves *Chrysocyon brachyurus* (Wasser *et al*, 2009) and humans (Kalmus, 1951; Brisbin & Austad, 1991; Settle *et al*, 1994; Schoon & De Bruin, 1994; Schoon, 1996; Schoon, 2005; Jezierski *et al*, 2010).

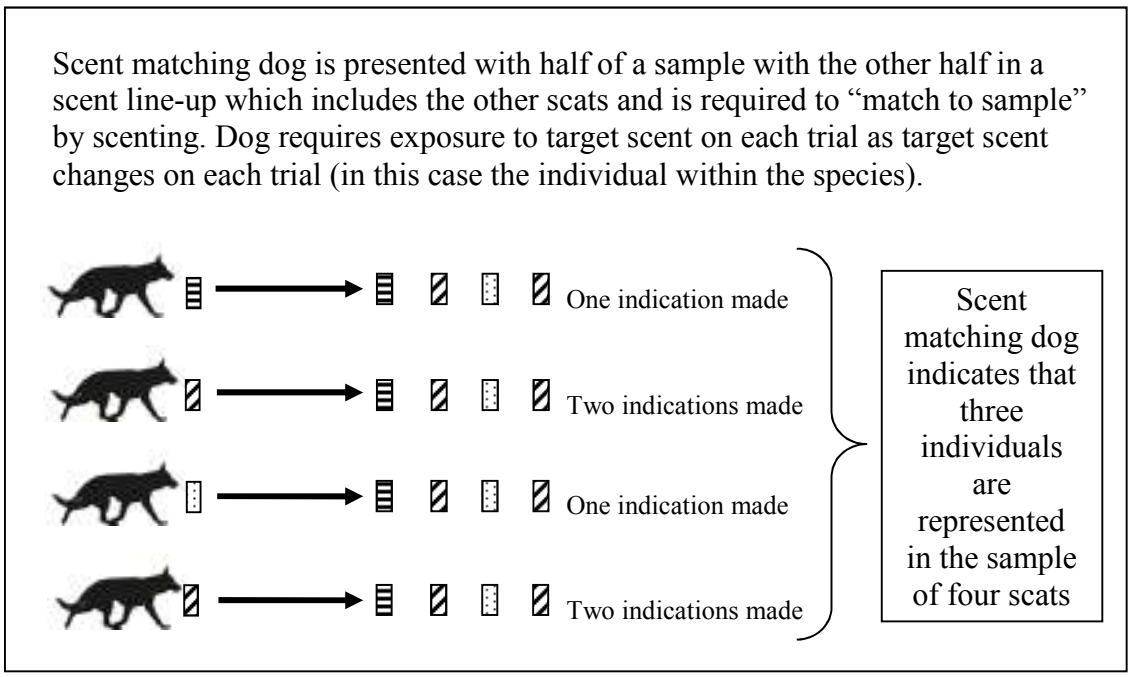

Figure 2. Graphic illustration of scent-matching dog concept in a hypothetical experiment to establish number of individuals within a sample of same-species scats collected from a survey area.

In scent-matching, the target scent is normally changed at every trial so a long-term olfactory memory is not desired. Typically in these studies, the dog is presented with a scent-producing item and then tasked to match this sample via olfactory means to a sample in a line-up of samples. For example, a researcher could present a scent-matching dog with the scat of a particular individual (target scat). The dog samples the scent from this target scat and then proceeds to locate a scat (within a line-up of scats including other individuals of that species) which matches the target scat. A portion of the target scat is normally included in the line-up, so the dog is provided with the option of a correct match. Scats from other individuals of the same species are also included in the line-up. If the trained dog indicates a match more than once in a trial with those samples, the researchers could conclude that the target individual is represented more than once in that line-up. Systematically repeating this procedure with all of the scats collected from a survey area allows researchers to establish how many individuals are represented in that population. Results showed that in terms of discriminating individuals within a group sample, dogs were similar in terms of accuracy with faecal DNA individual genotype testing and in some instances outperforming genotype testing when samples were too degraded for genotype testing to be performed (Wasser *et al*, 2009).

1.3 Scenting Vectors

Scent dogs can be tested either directly using the scent-emitting item, such as scat (Smith *et al*, 2003; Kerley & Salkina, 2007; Wasser *et al*, 2009; Fukuhara *et al*, 2010), or via a second item, which has been in direct contact with the scent-emitting donor, i.e. scent transfer has occurred from the donor to this second item (Kalmus, 1951; Brisbin & Austad, 1991; Settle *et al*, 1994; Schoon & De Bruin, 1994; Schoon, 1996; Schoon, 2005; Jezierski *et al*, 2010) (Table 1). I define (and hereafter use) the former as "direct vector" and the latter as "proxy vector" methods of scent-matching and an item to which scent is transferred as a "proxy".

Table 1. A list of studies using domestic dogs *Canis lupus familiaris* to scent-match, detailing scent donor species, vector used for scenting (direct from scent-producing item or from a proxy which has been in contact with scent-producing article) and description of scenting vector.

Scent Donor Species	Scenting Vector		References
	Direct	Proxy	
Humans *Homo sapiens*		Handkerchief	Kalmus (1951)
		Leather and metal dumbbell	Brisbin & Austad (1991)
		Cotton cloth	Settle *et al* (1994)
		Stainless steel tube	Schoon & De Bruin (1994)
		Stainless steel tube	Schoon (1996)
		Stainless steel tube, PVC electrical tubing and cotton cloth	Schoon (2005)
Amur tigers *Panthera tigris altaica*	Scat		Kerley & Salkina (2007)
Maned wolves *Chrysocyon brachyurus*			Wasser *et al* (2009)
Humans *Homo sapiens*		Cotton cloth	Jezierski *et al* (2010)

The proxy vector has been utilised by both detection dogs and scent-matching dogs. An example of this application with detection dogs is provided by Walczak *et al* (2012) where dogs were trained to detect cancers (breast cancer, melanoma and lung cancer) from breathe samples, which had been exhaled onto an absorbent cotton proxy by the human patient scent donors. In this study, the dogs did not make direct contact with the actual scent-emitting

source (human donor), but performed the detection work on a proxy which had been in direct contact with the human donor. In scent-matching applications, various studies (Kalmus, 1951; Brisbin & Austad, 1991; Settle *et al*, 1994; Schoon & De Bruin, 1994; Schoon, 1996; Schoon, 2005; Jezierski *et al*, 2010) describe how dogs are able to match individual human scent via a proxy, such as a cotton cloth or metal tube.

Scent-matching of non-human biological applications has only been documented in two studies (Kerley & Salkina, 2007; Wasser *et al*, 2009) and in both of these studies only the direct vector method was used, i.e. the dogs were presented with an actual scat (fresh or freeze dried) and required to match this scat with a scat in a line-up.

While the scope of scent-matching is vast, using actual samples does present limitations. Samples are not always easy to transport to a facility where scent-matching can be performed, samples degrade and may also require permits to cross international borders. Furthermore, while removal of these samples from the natural environment is generally seen as "non invasive" (because it is thought not to disturb the target species) it can be invasive because it alters the environment from which it was removed. Notwithstanding the benign nature of a scat, the removal of a scat from a natural environment could theoretically be a disturbance both to the target species (e.g. by disturbing territorial scent-marking activities) and other species which utilise scat (e.g. coprophagous insects).

By using the proxy vector method within the scent-matching protocol detailed above, it could be feasible to train dogs to scent-match field samples using proxies, i.e. no actual samples are removed from the field, but scent is instead donated from the sample onto a proxy. If it is possible to do so, scent-matching by dogs could provide researchers with a relatively cheap, technologically uncomplicated and "least invasive" survey tool, which could also be exempt from acquiring export permits and logistical difficulties. A field researcher uncertain of the presence of an animal species in a survey area (where a visual confirmation of the animal has not been possible or the scat is not easily identifiable) could place a proxy on a suspected scat of the target species in question and then easily ship this field-collected proxy to a facility where scent-matching could be performed. A proxy gathered in a similar manner from a captive animal could then be collected and via authenticated scent-matching by dogs, the field-collected scent sample identified to species.

A similar procedure has already been employed in the training of detection dogs in situations where access to individuals of a donor species has not been possible, but detection dogs required operant conditioning to this species' scent prior to being deployed in the field to detect individuals of that particular species. For example, the scent of invasive brown tree snakes *Boiga irregularis* in Guam was transferred onto cotton swabs and shipped to North America where the detection dogs resided for their training. The dogs were then trained to detect brown tree snakes via the cotton swab proxy and were subsequently able to detect individuals of this species in the field without ever having been exposed to the actual species in their training (Parker M, *pers comm*).

Scent-matching by proxy could also provide a useful tool for olfaction studies exploring whether animals do emit discernible odour. This is especially so when it is not viable to utilise the actual scent-emitting item, i.e. the actual animal under examination, for reasons of safety (dangerous study species) or logistics (species cannot be removed from field for examination).

Olfaction plays a major role in the lives of many animals (Sommerville & Broome, 1998; Campbell-Palmer& Rossell, 2011). Sommerville & Broom (1998) note that it is perhaps the limited olfactory ability of humans that restricts our scientific enquiry in this field. Harnessing the olfactory acuity of dogs, in a manner which has fewer limitations than the direct vector methods currently employed, could provide three benefits. Firstly, this could be a very useful tool in the under-examined field of olfaction studies. Secondly, a convenient research tool could be made available for both field and laboratory-based researchers who do not have access to the olfactory acuity of dogs. Thirdly, qualities of olfactory stimuli, which we are unable to perceive, could become apparent through the careful use of a species which is able to perceive these qualities (Auffarth, 2013).

1.4 Earlier Studies

The goal of my study was to develop a new application in scent-matching in dogs by matching scent from multiple donor species via a proxy. There are no published accounts which could be directly compared to my study; other studies used several individuals of one species. However it was helpful to consider these previous studies in this general field of study, i.e. training of scent dogs *per se*. Within these earlier studies, there exists a number of areas to be considered in view of technique improvements and modifications, namely:

training accounts; methods of training; modality use; sample arrangement; inter-sample distance; sample size and scenting vectors. This subset of factors was identified through suggestions made in the literature (mentioned in the following section) and also in my experience as an animal trainer.

1.4.1 Training Accounts

Detailed accounts of the training of dogs in olfactory tasks are lacking (Schoon & De Bruin, 1994; Schoon, 2005; Jezierski 2010; Hall *et al*, 2013) which may be due to the fact that scent dogs are generally used as a means to measure another problem under investigation (e.g. population surveys, occurrence of individuals within a population, proxy vector methodology) and the training of the dogs is thus of secondary importance. This omission of training accounts is nonetheless surprising considering the potential benefits of this tool.

1.4.2 Methods of Training

A stimulus that increases the likelihood of a particular behaviour occurring in the future is termed a reinforcer and animals will display choices which result in increased incidences of these reinforcers (Mondadori *et al*, 2005). Animal training requires that the test subject is motivated to not only perform a particular behaviour, but to repeat this behaviour in the future - reinforcers are hence often utilised in animal training. Reinforcers are typically used in the training of scent dogs and have been utilised in various forms, e.g. food (Kerley 2004; Jezierski *et al*, 2010; Fischer-Tenhagen *et al*, 2011: Walczak *et al*, 2012), toy/play (Schoon, 2005; Richards *et al*, 2008; Wasser *et al*, 2009), praise from the handler (Brisbin & Austad, 1991) or food and praise (Settle *et al*, 1994).

In scent-matching training, there have been two different approaches in the initial stages of training. In some studies, the dogs are first trained to visit the stations which will ultimately house the samples available to the dog (Kerley 2004; Jezierski *et al*, 2010). In other studies, the dog is not specifically trained to visit all of the stations, but is rather taught that a matching scent is associated with a reinforcer (Settle *et al*, 1994; Wasser *et al*, 2009). In the latter approach, the trainers rely on the dog's ability to sustain a search for the matching scent, which should involve a visit to each station until the matching sample is located.

1.4.3 Modality Use

Schoon (1996) noted that the initial training of dogs should be done according to the eventual task required, so if a dog is being trained to ultimately use scenting as the means to performing an exercise, other "information modalities" such as vision should be excluded right from the initial stages of training to ensure that scenting is the only modality available to the dog. Of the nine scent-matching studies referred to in this project (Table 1), eight reflect this concept of excluding visual aids from the start of training. Two studies excluded visual aids by blocking visual access altogether through the use of solid containers (Kerley & Salkina, 2007; Wasser *et al*, 2009) and three studies excluded visual aids by using identical non-target decoys from the initial stages of training (Kalmus, 1951; Brisbin & Austad, 1991; Jezierski *et al*, 2010). Three studies using police dogs (Schoon & De Bruin, 1994; Schoon, 1996, 2005) do not describe the training of the dogs, so it is unknown whether visual aids were excluded from the initial stages of training, but the main author of these studies advocates the exclusion of non-scent sources of information from the initial stages of training, so it is possible that this principle was adhered to in these studies. Only one study (Settle *et al*, 1994) used visual aids, but only in the very early stages of training, i.e. the scent target was visually apparent to the dogs, but only in the very initial stages of training.

1.4.4 Sample Arrangement

Various permutations on the arrangement of the samples presented to the dog for olfactory sampling have been used – in some studies the scented items was placed directly on the floor, either side by side (Brisbin & Austad, 1991) or in a straight line (Kalmus, 1955; Oesterhelweg *et al*, 2008; Schoon & De Bruin, 1994; Settle *et al*, 1994; Schoon, 1996). In other studies, the scented article was placed within a container and then placed into a non-transparent holder in a straight line (Wasser *et al*, 2009), arc (Jezierski *et al*, 2010) or a circle (Schoon, 1996; Kerley & Salkina, 2007). Wasser *et al* (2009) reported that subjects over-ran samples in the straight-line set-up due to their enthusiasm and commented that modifying the straight line line-up into an oval may minimise this problem. Most recently a "sled" device, where the dog remains on a platform and visually-identical samples are presented via a remote sliding mechanism, bringing the samples within the dog's olfactory sampling proximity, has been used by Fischer-Tenhagen *et al* (2011).

1.4.5 Inter-Sample Distance

Distances between samples are not always specified in the literature, but where specified, distance ranges from 40cm - 60cm (Schoon, 1996) to 85cm (Settle *et al*, 1994) between samples. Authors of these earlier studies do not provide reasons for these distances between samples, but it could be fairly safely assumed that samples are spaced reasonably far apart to avoid a presumed "scent overlap", i.e. the scent of one sample does not mingle (and presumably interfere) with the scent of another sample (Figure 3).

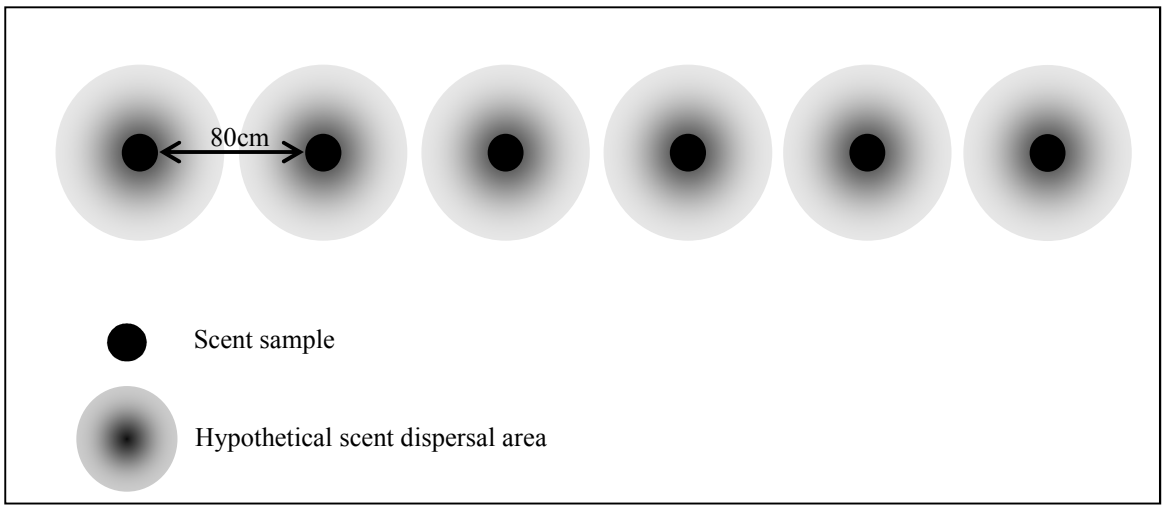

Figure 3. Hypothetical set-up of scent samples in a six sample scent-matching line-up to avoid scent overlap of samples by increasing inter-sample distance and straight-line arrangement.

1.4.6 Sample Size

Typically, studies of detection or scent-matching dogs involve very small samples (Table 2), which is mainly due to three reasons. Firstly, in some instances dogs are acquired specifically for the project (Kerley & Salkina, 2007; Richards *et al*, 2008; Wasser *et al*, 2009) and lifelong care once the project is complete is required, so a large sample is not desirable. Secondly, some studies use dogs already in police service (Settle *et al*, 1994; Schoon & De Bruin, 1994; Schoon, 1996, 2005) and the scent work is secondary to the service obligations of the dog, which means that some dogs are not able to complete the training due to other responsibilities. Thirdly, dogs sourced from welfare institutions have been used in scenting application, citing a "high drive" as an integral part of being a successful scenting dog (Wasser *et al*, 2009). Sourcing "high drive" dogs purely for a study does however pose logistical problems (housing, transport), financial problems (food and veterinary care) and

ethical problems, such as enrichment when not being trained and the fate of the dog once the study is complete.

Table 2. Review of studies (sample sizes and source of subjects) using domestic dogs *Canis lupus familiaris* to detect or match scents.

Source of Subjects	Sample		References
	Start	End	
Companions	3	1[1]	Brisbin & Austad (1991)
Police services	10	7[2]	Settle *et al* (1994)
Police services	6	6[3]	Schoon & De Bruin (1994)
Police services	8	8[3]	Schoon (1996)
Specifically acquired	7	5	Kerley (2004)
Police services	11	11	Schoon (2005)
Specifically acquired	2	1	Richards *et al* (2008)
Specifically acquired	3	3	Wasser *et al* (2009)
Specifically acquired[4]	6	6	Jezierski *et al* (2010)
Companions	7	6	Fischer-Tenhagen *et al* (2011)
Specifically acquired[4]	6	3	Walczak *et al* (2012)

[1]Only one subject participated in all six treatments

[2]Not all subjects able to complete training due to conflicting obligations

[3]Trials unequal between subjects due to workload, illness and death

[4]Not overtly stated, but subjects housed in kennels and not police dogs; therefore assumed that subjects specifically acquired for project

Privately owned, i.e. companion dogs, are however capable of being trained to detect a target scent (Fischer-Tenhagen *et al*, 2011). When working with companion dogs, husbandry requirements are virtually non-existent, there are no ethical conflicts in terms of supply or retirement of the dogs after the project and there is a vast supply of suitable candidates. Furthermore, the dog owners (handlers) normally have some experience of animal training, which reduces the amount of time and effort required to brief the handlers on their function in the training process.

1.4.7 Scenting Vectors

Scent-matching studies conducted to date have utilised proxies (Kalmus, 1951; Brisbin & Austad, 1991; Settle *et al*, 1994; Schoon & De Bruin, 1994; Schoon, 1996, 2005; Jezierski *et al*, 2010) involving only humans as scent donors in all of these studies. To date, there has been no study of the training of dogs to scent-match via proxy from non-human scent donors. Furthermore, all scent-matching via proxy studies have been restricted to donors within a species and no studies have addressed scent-matching via proxy with scent donors of multiple species.

1.4.8 Signal Detection Theory

Signal detection theory considers measurement of stimuli over behaviour (Davison & Tustin, 1978). In the application of scent-matching, the stimulus (e.g. scent presented to the dog) can be measured in terms of the dog's trained response (behaviour) to that stimulus. While signal detection theory is pertinent to scent-matching, it has not been a major area of focus in previous studies on scent-matching by dogs. This is possibly due to the author's focus being on the data generated from scent-matching as opposed to the mechanisms involved in scent-matching.

Signal detection theory has a concise set of terminology relating to the whether the stimulus is present or absent and whether or not a response occurred. This terminology is used as follows: stimulus present with a response is a "hit"; stimulus present with a non-response is a "false rejection/miss"; stimulus absent with a response is a "false alarm" and stimulus absent with a non-response is a "correct rejection" (Davison & Tustin, 1978). In previous studies of scent-matching by dogs, a variety of terminology is used, e.g. a "hit" is described as a "correct choice" (Settle et al, 1994), "correct response" (Jezierski et al, 2010) and "correct recognition" (Schoon, 2005).

1.4.9 Sensitivity and Specificity

Previous studies of scent-matching via proxy addressed both sensitivity (can the dogs detect a human donor scent) and specificity (can the dogs discern or generalise human donor scents) (Settle, *et al*, 1994; Schoon, 2005; Jezierski *et al*, 2010).

1.5 Proposed Modifications

Based on a review of the earlier studies, I believed that modifications to previous training protocols and the devices used could enable the training of a unique task in dogs, i.e. to scent-match among species via proxy. Being able to utilise a verifiable method of scent-matching could be of benefit to researchers investigating olfaction theories as well as to commercial and civil activities where detection dogs are trained and scent categorisation needs to be explored, i.e. can a dog be trained only to detect a particular scent (e.g. particular termite species) or can a dog be trained to detect a category of scents (e.g. wood boring insects)?

1.5.1 Modality Use

In previous studies of scent-match training in dogs (Table 1), visual modalities were restricted, with the idea that the use of any other modalities of information (e.g. visual stimulus) would be counter-productive to the end goal of only olfactory discrimination (unimodal stimulus), i.e. the dogs were required to use only their sense of smell to discriminate between samples, so any other modality was excluded from the initial stages of training (Brisbin & Austad, 1991; Kerley & Salkina, 2007; Wasser *et al*, 2009; Jezierski *et al*, 2010). While this unimodal training approach has produced results, it is worth considering whether using more than one modality of information during training could perhaps produce better results.

Presenting the animal with two different modalities of information (e.g. visual and olfactory) results in a compound bimodal stimulus, which speeds up learning (Rowe, 2005). It is debated as to whether the animal perceives the compound as a one unit of very useful information or as two units of useful information, although Kehoe *et al* (1994) suggest both possibilities are at work. Nonetheless, it is clear that compound bimodal stimulus results in an additive positive effect on learning (Rowe, 2005).

In my study, two modalities, visual and olfactory, were combined to create a compound bimodal stimulus. The risk when using bimodal stimulus however is that once the stimuli are separated and one stimulus is not presented, the animal's learning may be negatively affected. In other words, if two modalities such as vision and olfaction are used in training, will the animal still be able to perform the task if visual stimulus is removed? I believed that moving from a compound bimodal stimulus in early training to a unimodal stimulus in later training would not negatively impact the learning process of the dogs, for two reasons. Firstly, Rowe

(2005) reports that animals trained using a compound bimodal stimulus respond just as well to one of the isolated stimulus later because the associative action has already been established with each stimulus. Secondly, the salience of the stimulus impacts on the associative strength with that stimulus in the future, i.e. the more conspicuous a stimulus is to an animal, the stronger the associative link will be to that stimulus in the future (Rowe, 2005). It has been stated that depending on the context, dogs can alternate between the modalities of olfaction and vision with olfaction thought to be the predominant modality (Gazit & Terkel, 2003a; Szetei *et al*, 2003). Therefore, I believed that using a compound bimodal stimulus (visual and olfactory) in early training and then removing the less salient stimulus (visual) once an olfactory association has been made by the dog, would maximise learning.

The efficacy of a compound bimodal stimulus in training a complex olfactory task in dogs was not tested *per se* in this study, because the goal of my study was first and foremost to establish whether dogs could scent-match via proxy among species. This study could not examine what might enable the acquisition of scent-matching among species by dogs without knowing whether such a possibility existed in the first instance. While my assumption about the modality does form part of my motivation for the device used in the training of scent-matching among species, modality use is a broad topic of study which fell outside of the scope of this study and warrants singular investigation in future studies. The compound bimodal stimulus approach was used in this study merely to maximise learning.

1.5.2 Levels Of Training

The end goal of scent-matching via proxy requires the subject to establish a reference to a novel scent (sample) in a short space of time, move away from the handler, independently assess available sample options and then match the sample to one of the options provided, by offering a pre-trained response at the selected sample. This is a complex multi-sequenced task for a nonhuman animal. Furthermore, the task required that the subject was able to form a cognitive concept of the task (match-to-sample) and that this concept could be applied even when presented with a novel sample.

When training an animal to accomplish a complex task, Bailey (quoted by Enquist & Ghirlandia, 2005, pp 159) recommends breaking the end task down into smaller levels and making the goals of each level unambiguous to the animal, via differential reinforcement, i.e.

any behaviour which is closer to the desired behaviour is reinforced (rewarded) and any behaviour which is further from the desired behaviour is not rewarded. Bailey continues to state that each phase of training should consolidate and apply the learning which occurred in the previous level.

In a previous study by Jezierski *et al* (2010), the end training goal required the dog to scent-match an unknown human-scented proxy from five samples, all of which were scented by unknown humans (one being the target proxy and the remaining four being decoy proxies). The end task was decomposed into smaller tasks (which I refer to as sub-tasks), all the while working towards achievement of the end task. This concept is defined as successive approximation and is recommended for training of complex tasks (Krueger & Dayan, 2009). Three training phases were conducted with the dogs in the study by Jezierski *et al* (2010). In my study, the end goal was very similar to that of Jezierski *et al* (2010), with the only difference being multi-species target and decoy scents, with five decoys. I however used a total of nine levels of training compared to their three stages of training. The additional six levels in my study were achieved by reducing increments of difficulty in each of the sub-tasks which resulted in a greater number of sub-tasks, i.e. the end task was split into more sub-tasks and hence more levels.

My reasons for the threefold increase in levels of training was primarily because more levels of training allowed for smaller increments of learning between these levels, which should have made achievement of each level's success requirements easier. Kerley & Salkina (2007) do however note that the performance of scent dogs appears to decline if novelty is restricted in the samples presented to the dog for scent-matching. Of the nine training levels I proposed in training scent-matching, the target scent in the first six levels remained identical, i.e., handler scent. A possible negative effect of increasing the levels of training as I proposed (and only introducing a novel target scent after two thirds of the training levels had been completed) could have been boredom and possible decline in performance as a result. I did however believe that the benefits of having a high number of training levels would outweigh the risk of boredom.

Dividing the task into sub-tasks was accomplished using a human construct of olfactory perception, i.e. humans cannot perceive the scent on the proxies, so assumptions were made as to what scent would be present/absent on the proxies. This created a difficult situation

where an ignorant perceiver applied a perception criterion to a more perceptive species. This difficulty was not unique to my study as olfaction typically presents this type of perceptual property problem to researchers (Auffarth, 2013). In trying to minimise such "ignorant perceiver" problems, sub-tasks were determined with as few assumptions as possible and sub-tasks as previously used in similar studies (Settle *et al*, 1994; Jezierski *et al*, 2010) were used as a guide.

1.5.3 Sample Arrangement

Wasser *et al* (2009) note that a straight line set-up of samples is associated with subjects over-running samples due to enthusiasm. I agreed with the author's recommendation that an oval design could reduce the problem of over-running samples. I therefore used a circular design – with no obvious beginning or end – for the arrangement of samples in scent-match training of dogs.

1.5.4 Inter-Sample Distance

While not always explicitly stated, it is assumed that the distance between samples in previous studies (min. 40cm, Schoon, 1996) was motivated by the desire to avoid assumed scent overlap of samples (Figure 3). I contended that deliberately creating a scent overlap, by placing samples closer together than previously done (i.e. < 40cm) and by using a circular arrangement of these samples (Figure 4), could be beneficial to the acquisition of scent-matching.

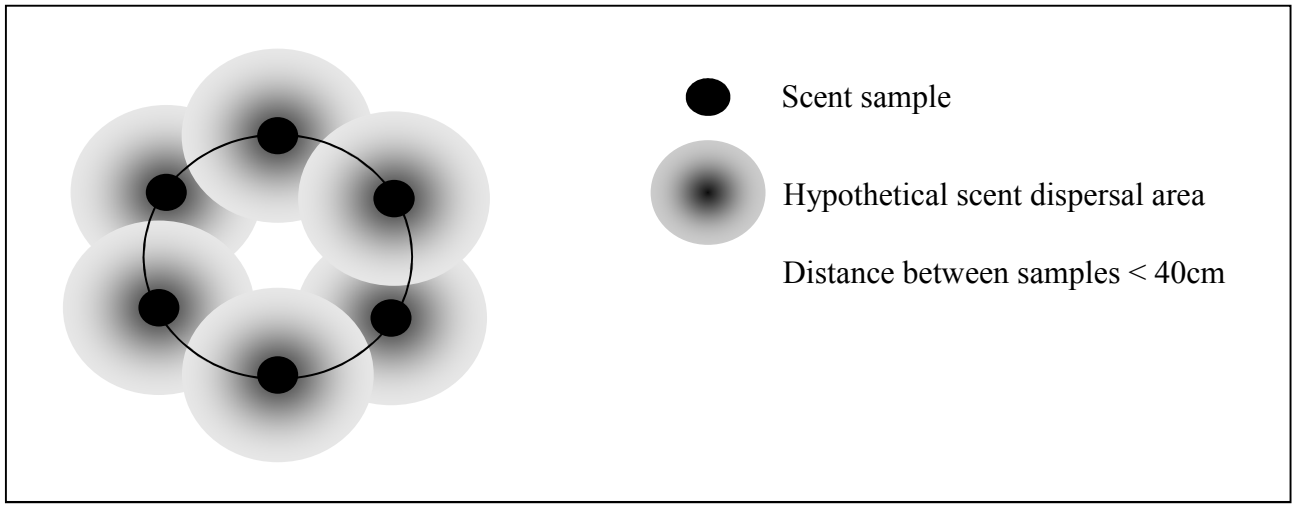

Figure 4. Hypothetical set-up of scent samples in a six sample scent-match line-up to cause probable scent overlap of samples via closer inter-sample distance and circular arrangement.

I had five reasons for why deliberately creating a scent overlap would lead to success in scent-matching training with dogs. Firstly, it is not known from what distance a dog can conduct olfactory sampling or how far the scent from an item spreads out to create an odour plume. Therefore, it is possible that despite the attempts made in the past to prevent scent overlap this could have occurred in any event. It is however widely accepted in the dog training community that dogs are capable of "scent-filtering" (the ability to filter out non-target scents and identify a target scent), so even if scent overlap occurs, a dog should be capable of filtering out the non-target scents. Secondly, if scent overlap does not occur, any alternate option/s are not apparent from the start of olfactory sampling. In other words, being aware of multiple options simultaneously (due to the scent overlap) could reduce the possibility of the dog making a "close enough" match, instead of sampling all options available. Thirdly, some authors state that the performance of scent dogs improves when presented with novelty (Kerley & Salkina, 2007). Creating a scent overlap could produce a novel situation depending on the combination of scents involved and thereby increase the performance of the scent-matching dog. Fourthly, a smaller sample placement area will result in less physical exertion by the dog and result in greater endurance during scent-match training, which is associated with better performance in scent tasks (Gazit & Terkel, 2003b). This increased endurance should allow for more trials to be run within a training session and hence provide more opportunities for beneficial associations (reward) during such a session. Fifthly, samples in close proximity to one another should enforce a more thorough olfactory sampling style from the dog i.e. a "deep sniff" of the sample to ensure that the sample is indeed the source of the target scent and not a vagrant scent from another sample. For these reasons, I reduced the distances between samples when compared to earlier studies (< 40cm) and thereby deliberately created an assumed scent overlap.

1.5.5 Signal Detection Theory

Due to the variety of terminology used in previous studies and the fact that my study did not involve a "stimulus absent" treatment – thereby negating the possibility of a "correct rejection" or "false alarm" – I chose to use a simpler terminology of "match" or "non-match".

1.5.6 Sensitivity and Specificity

Due to the fact that my study was unique (it was not known whether dogs could scent-match non-human scent donors via proxy) my study only considered sensitivity and not specificity because sensitivity needs to be established before specificity can be addressed.

1.6 Aims and Objectives

My study aimed to establish whether domestic dogs *Canis lupus familiaris* are able to discriminate a scent sample from a donor among a group of samples from donors of other species (with all samples being scented proxies) through the use of an expanded training programme and unique device.

My objective was therefore to develop and authenticate a training programme for dogs to scent-match via proxy among scent donor species and to keep detailed records of this training.

I hypothesised that dogs are capable of scent-matching among donor species, via a proxy, with my prediction being that eventual success rates would be significantly greater than chance ($p < 0.05$). I further hypothesised that when compared to a computer-simulated untrained dog, a trained dog would have a higher success rate in scent-matching among donor species via proxy than the computer-simulated untrained dog and I predicted that this difference would be statistically significant ($p < 0.05$)

Chapter 2: Materials and Methods

Over a period of 13 months, 15 subjects (split into three training groups) commenced the training programme (Table 3). Ages of subjects were rounded to the closest whole number in years. In the instance of cross-breed dogs, the breed was listed as a "type". Twelve of the subjects completed this training. The three subjects which did not complete the training were ZC and OS (subjects did not meet training criteria) and MG (chronic health problems).

Table 3. List of domestic dog *Canis lupus familiaris* subjects detailing training group, subject name, age at start of training, breed, sex, reproductive status and training period during scent-match training.

Training Group	Subject Name	Age at start of training	Breed	Sex	Reproductive Status	Training Period
1	ZR	2 years	Dobermann	Female	Sterilised	November 2012 – June 2013
	NM	8 years	Border collie	Male	Sterilised	
	FF	5 years	Dachshund type	Female	Sterilised	
	ML	6 years	Labrador retriever	Female	Sterilised	
	ZC*	3 years	German shepherd	Male	Intact	November 2012 – January 2013
	MG*	6 years	Labrador retriever	Female	Sterilised	
2	SC	5 years	Labrador retriever	Female	Sterilised	April 2013 – December 2013
	EM	5 years	Springer spaniel	Female	Sterilised	
	CH	5 years	Flat-coated retriever	Male	Sterilised	
	HP	2 years	Labrador retriever	Female	Sterilised	
3	OS*	4 years	Border collie type	Male	Sterilised	April 2013 – October 2013
	BB	3 years	Border collie type	Male	Sterilised	April 2013 – December 2013
	PC	2 years	Labrador retriever	Male	Sterilised	
	CR	2 years	German Shepherd dog type	Male	Sterilised	
	VC	1 year	Border collie type	Male	Sterilised	

* Removed from the programme

A total of 93 training sessions were conducted. Two sessions for Group 3 were cancelled due to inclement weather (excessive wind) and were rescheduled. Subject SC was the only subject to attend all sessions. None of the other subjects attended all sessions due to other commitments on the part of the handlers or poor health. A total of 4 082 runs were conducted across all levels of training for all training groups. An average of three trials was conducted in each session, each comprising an average of five runs per trial.

2.1 Study Subjects

I used a pool of companion dogs recruited from dog training schools in the greater Johannesburg area. Criteria for inclusion were: some basic training of the dog (walk on lead, sit/lie down on cue); the dog was comfortable in the presence of other dogs and people (both known and unknown); the dog was willing to accept a reward in the form of food or a toy; handlers were prepared to follow instruction and refrain from scent training outside of the training sessions in this study; and, notwithstanding extreme unforeseen circumstances, handlers were committed to completing all of the training and testing in this study. Criteria for exclusion, at the start of and during experiments, were any chronic health problems in the dog (e.g. allergies, orthopaedic problems) or unfeasible temperament in the dog (e.g. aggression, timidity).

While companion dogs do have advantages in a study such as this (e.g. large pool of candidates, low cost, no long-term care) a possible drawback to using companion dogs as study subjects is that many variables (e.g. breed, age, sex, reproductive status, past training history, home environment) cannot be controlled, i.e. these are "nuisance variables" (Kirk, 2012, p24). Nuisance variables were recorded and allocated across groups of dogs being trained, e.g. where a difference existed (such as sex or breed) all attempts were made to ensure that each group had an equal representation of male and females and that breeds were represented in each group. The influence of these variables on training was examined statistically where possible.

2.2 Materials

2.2.1 Proxies

Dogs are capable of detecting the scent of an individual human from an item which has been in contact with that individual (Kalmus, 1951; Settle *et al*, 1994; Schoon, 2005; Jezierski *et al*, 2010). In my study, all scent was transferred onto such an item, which was termed a

proxy. Proxies were visually identical, thereby ensuring that non-olfactory cues were absent as far as possible and that the donor scent on the proxy was the only information source available to the dog.

The proxy in this study was a 20cm x 20cm light beige woven cotton cloth as described by Settle *et al* (1994), Schoon (2005) and Jezierski *et al* (2010). Prior to use all proxies were machine washed in a standard domestic washing machine, mechanically dried and then sealed in an air-tight container until use. Once a proxy had been used (either as a scented proxy or a blank decoy proxy) the proxy was discarded until it had been cleaned and stored as described above. All proxies were handled with metal tongs and/or while wearing powder-free latex gloves, which were discarded at the end of a session.

2.2.2 Proxy Storage

Donor scented proxies were stored in 352ml transparent glass jars (Consol: product number 191551) during training to minimise scent contamination of the environment by the proxy and likewise scent contamination of the proxy by the environment as far as possible. These transparent glass jars were each labelled to ensure that the tester was aware of the contents, including labelling of blank decoys.

Once a jar had contained a proxy, the jar was discarded from that session i.e. the proxy that was contained in the jar would presumably leave a scent residue in the jar, thereby soiling the jar and rendering it useless to contain another proxy in that session. Newly purchased jars were washed prior to first use. After use labels were removed and jars were washed in a domestic dishwashing machine at 50°C using a commercial dishwasher detergent. Jars were then air dried, sealed and stored prior to use. After washing and during training sessions, jars were only ever handled by the tester or the tester's assistant using powder-free latex gloves.

2.2.3 Scent Wheel

To combine the features of a bimodal information system, circular presentation of samples and reduced inter-sample distance, I designed a "scent wheel" (Figure 5) – a wire circle (60cm height X 75cm diameter) which had six holders (18cm height X 9cm diameter) placed approximately 38cm apart.

Figure 5. Scent wheel devised for training of scent-matching via proxy in domestic dogs *Canis lupus familiaris* showing holders and holder number markers.

Holders were loosely connected to the wheel via small plastic rings, which allowed the holder to be slid up to place a jar underneath the holder (Figure 6). Each holder had a wire mesh over the top of the holder to allow the subjects to sample the scent of the proxy within the jar contained in the holder without disturbing the jar or the proxy. A number card above each holder indicated the holder position. Holders were constructed from sturdy wire to prevent the subject from making physical contact with both the jar in the holder and the proxy contained within the jar.

Figure 6. Close up view of holder on a device designed to train scent-matching via proxy in domestic dogs *Canis lupus familiaris* from front, top and back showing jar placement in holder from front and top angle.

The subjects did make tactile contact with the scent wheel during a session. The scent wheel was not cleaned during a session though, because preliminary data showed that tactile contact with the scent wheel by the subject neither influenced future performance by that subject nor of the other subjects working on the scent wheel in that session. The scent wheel was cleaned immediately after each training day by lightly spraying the entire device with a commercial odour remover (F10 Odour Eliminator, manufactured by Meadows Animal Healthcare United Kingdom) and then left to air dry in an indoor location.

2.2.4 Table

A small plastic table (Figure 7) was used in the pre-training of the subjects to partially obscure the target proxy/jar by placing the proxy/jar on the inside of one of the table feet.

Dimensions:
Height 490mm
Width 790mm
Length 790mm

Each foot of the table served as a potential concealment area

Figure 7. Plastic table used in early levels of scent-match training in domestic dogs *Canis lupus familiaris* to partially conceal a scented item, detailing dimensions and concealment areas of table.

2.3 Training

2.3.1 Sessions

In each level of training, the handler (person responsible for the care of the subject) and the tester (person responsible for all management of the training sessions) were present. In some sessions, an assistant was also present to assist the tester in placing samples. Subjects were trained in groups with no group exceeding six subjects. This was done to keep a session to a reasonably short time to accommodate other commitments of the handlers and to minimise

waiting time between trials for each subject (and hence reduce the risk of boredom in the subjects).

One of three venues was used for the training and testing. All pre-training occurred in an outdoor venue (grassed area approximately 20m X 20m). Subsequent training and testing then occurred in either an indoor venue (hall approximately 15m X 20m with a concrete floor) or an outdoor venue (one walled roofed area approximately 8m X 5m with a wooden floor).

Dogs appear to acquire a task in a shorter number of sessions if those sessions are spaced a week apart compared to daily training sessions, possibly due to a longer period being available for consolidation of learning between training sessions (Demant *et al*, 2011). Subjects thus received a training session once a week. Figure 8 provides an overview of the run, trial and session composition in those sessions. The weekly training session per group was approximately 90 minutes in total, with each subject being trained for approximately 15 minutes in a session. In all instances, subjects were trained individually. Each subject underwent a training trial and then a rest (away from the training area). The next subject then underwent a training trial until each subject had completed that trial, after which the next trial started. The trial order of the subjects during a session was randomised for each session. In the earlier training levels, subjects undertook two to three trials of five runs each in a session and thereafter a maximum of five trials per subjects was conducted in any one session.

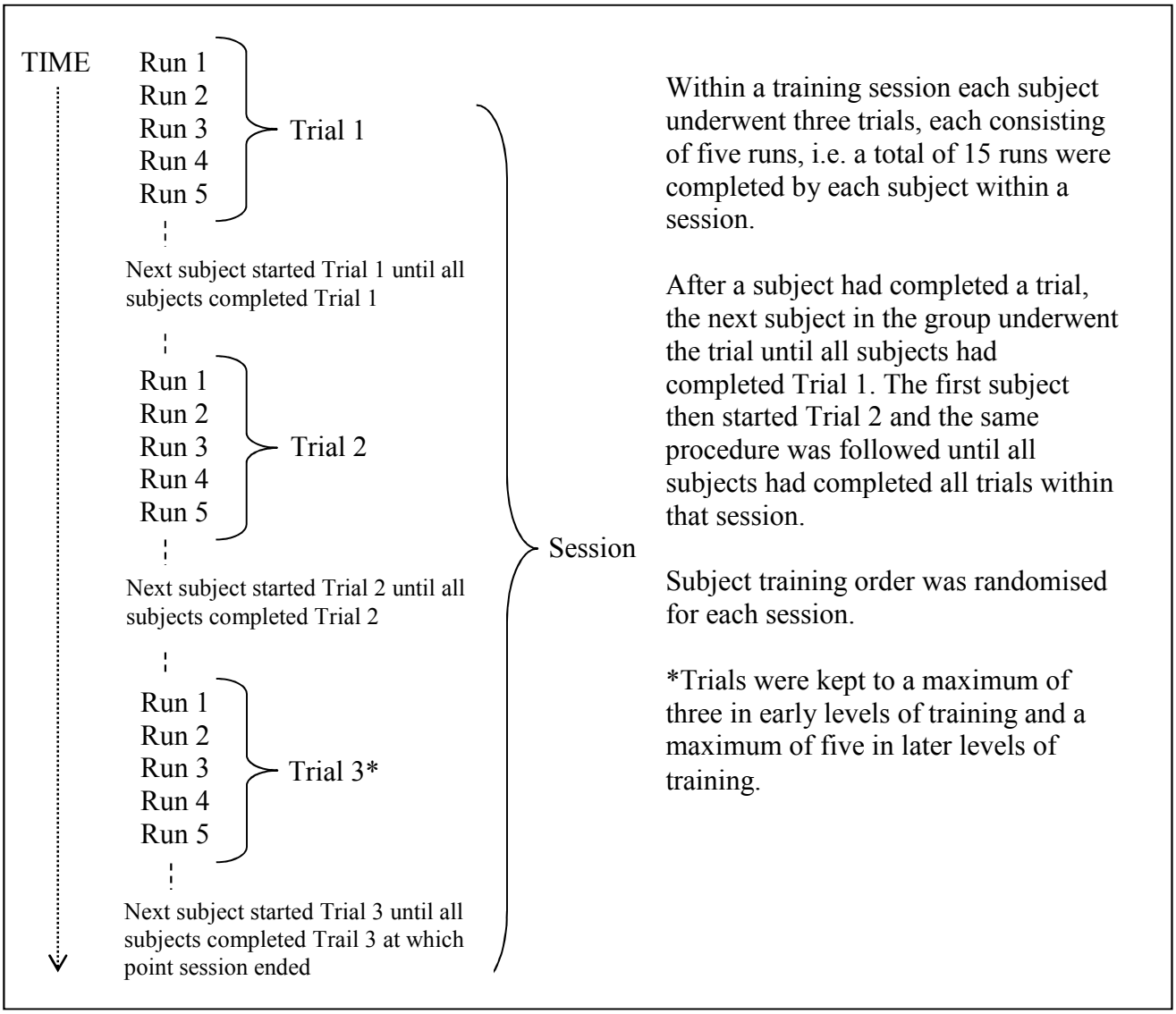

Figure 8. Breakdown of the experimental design showing runs and trials of a subject within a session of early scent-match training in domestics dogs *Canis lupus familiaris*.

On some occasions, the number of runs within a trial were reduced depending on the subject's performance, i.e. if the subject was performing poorly (no inclination to participate in the training in conjunction with more than three consecutive non-successful attempts to achieve a run objective), the trial was aborted before five runs had occurred (normally after three "poor performance" runs). General session data included date, venue of training, duration of training, training order of subjects, number of runs per subject, ambient training conditions and any exceptional information (e.g. subject appearing unwell).

Any subject which was suspected to be unwell was immediately excluded from training and a veterinary report was required from the handler prior to the subject's re-admission at the

following session. This occurred twice with two subjects (ML ear infection and CR gastroenteritis). Any data from these subjects collected prior to their exclusion in that session was included in the results for that session.

Subjects were provided with water and protected from extremes of weather. Handlers were requested to keep to the subject's regular routine on training days, i.e. normal meals and husbandry procedures. Reward type (food or toy) during experiments was at the discretion of the handler. A record of the reward used for each subject was kept by the tester. In the event of any major change to the working environment (e.g. replacement handler, different venue, repairs to device), I planned to subject the dogs to a priming trial i.e., a warm-up, but no priming trials were required.

2.3.2 Training Terminology

As per the example in Jezierski *et al* (2010), the terms used in the description of Training are described in Table 4.

Table 4. Glossary of terms used to describe the special training elements and procedures in training scent-matching among donor species via proxy by domestic dogs, *Canis lupus familiaris*.

Term	Description
Aged scent	Proxy used > 12 hours after scent transfer from donor
Blank decoy	A cotton cloth (proxy) which had not been in contact with a scent donor
Cue	The verbal command from the handler to the subject to match the reference proxy to a target proxy in the sample line-up
Decoy jar	The jar which contained a blank or scented decoy proxy
First attempt match	Subject matched the reference proxy to the target proxy on the first attempt
Fresh scent	Proxy used < 12 hours after scent transfer from donor
Giving scent	The handler presented a scented proxy to the subject and allowed the subject to perform olfactory sampling of the proxy
Indication	A clear response of the subject as to its choice of the target proxy e.g. sitting, lying down or pawing at the proxy
Match	Subject correctly matched the reference proxy to the matching target proxy in the line-up
Non-attempt	Subject did not sample the line-up of proxies
Non-match	Subject incorrectly matched the reference proxy to a decoy proxy in the line-up
Re-cast	Subject re-sampled the reference proxy after a non-attempt or a non-match
Reference proxy (RP)	The scented proxy presented to the subject at the start of a trial which matched a randomly placed scented proxy (target proxy) in the sample line-up
Reward	A food or toy delivered to the dog upon successful indication of the target proxy
Sample line-up	Five blank or scented decoy proxies and one randomly allocated target proxy (which matched the reference proxy presented at the start of the trial) were presented to the dog for olfactory sampling
Scented decoy	A cotton cloth (proxy) which had been in skin contact with a scent donor (human or non-human) or scent donor by-product e.g. scat and onto which scent from the donor/by-product had been transferred
Success rate	The number of first attempt matches divided by the number of runs within that level for that session
Taking scent	The subject performed olfactory sampling of a reference proxy
Target jar (TJ)	The jar which contained the target proxy
Target proxy (TP)	The scented proxy placed randomly in the sample line-up which matched the reference proxy presented to the subject at the start of a trial

2.3.3 End Process

While the actual training is described in detail later, an overview of the end run procedure is useful up front as it aids in understanding the goals and processes of the training (Figure 9).

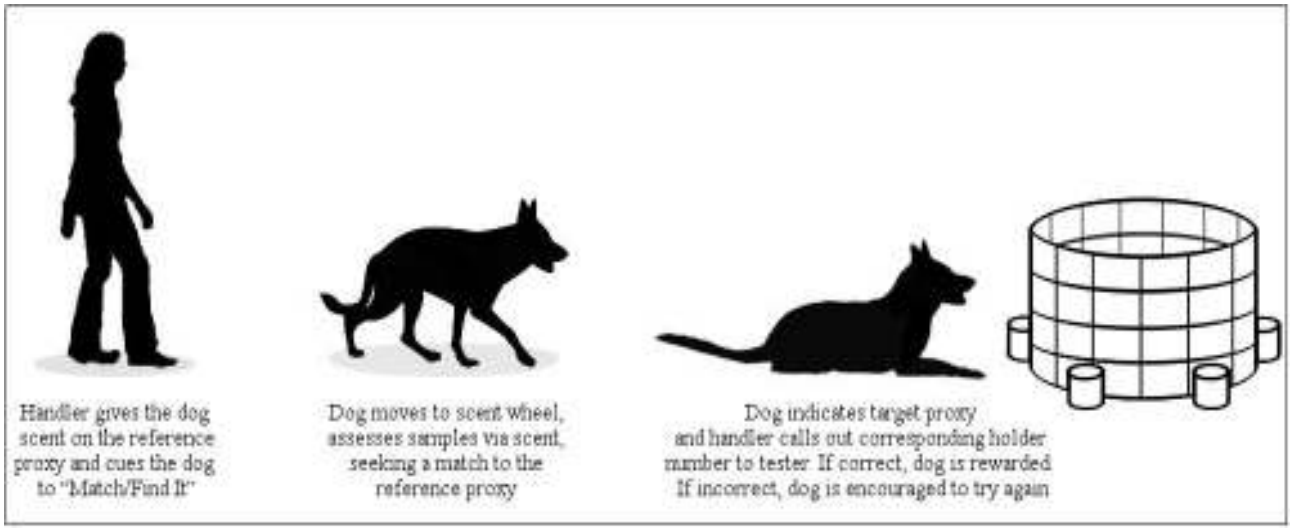

Figure 9. Graphic illustration of a run procedure during scent-matching via proxy by a domestic dog *Canis lupus familiaris*.

Two identical scented proxies – target proxy (TP) and reference proxy (RP) – were selected for a trial. Based on the subject's level of progress, the TP and RP were either scented by a known donor (known human) or a foreign donor (unknown human, other species, species by-product, such as scat, shed snake skin). The tester removed the TP from a sealed jar and resealed the jar. The TP was placed into a clean labelled jar, which was then placed into the scent-wheel (Figure 5) in a randomly allocated holder, corresponding to a preset random list of holders known only to the tester and assistant. The tester or assistant placed jars into all of the five remaining holders which (according to the subject's level of progress) were either empty or contained blank decoy proxies or scented decoy proxies. The tester then removed the RP from the sealed jar and handed the RP to the handler using a pair of metal tongs. The handler, wearing powder-free latex gloves, held the proxy lightly by a corner and allowed the subject to sample the RP. When the handler was convinced that the subject had done so adequately (demonstrated by the subject sniffing/mouthing the RP), the subject was released to perform the "match to sample" task on the scent wheel (Figure 5). The handler remained at a standard distance from the scent wheel (about 5m) and silent during this procedure so as to not direct or distract the subject. When the handler was convinced that the subject had made a definite choice (demonstrated by the subject lying down, sitting, barking or pawing at that sample) from among the six options available, the handler called out the holder number to the

tester. The tester answered “Yes” (match) or “No” (non-match). In the event of a match, the handler moved to the test subject and rewarded the subject with a food or toy reward. This was done as close as possible to the holder to re-affirm to the subject that this particular scent was associated with reward, without actually touching the holder (to prevent scent contamination of the holder). The subject was called away from the scent wheel and moved out of site of the scent wheel while the tester prepared for another run by moving the TP jar to a random holder in the scent wheel. In the event of a non-match, the handler either encouraged the subject to re-attempt the task by repeating the verbal cue to perform the scent-match, or recalled the subject for re-scenting of the RP (re-cast). Re-casts were counted but not recorded. If a subject re-casted more than three times during one run without making an attempt to match the sample, the trial was aborted and the subject was rested with this result being recorded. If the subject’s performance did not improve on the next trial with a different scented proxy, then the subject performed a short trial (three runs) on a lower training level (at which success was highly likely) and the subject’s session for that day was concluded. In the following session, the subject was started on a level in which competence had been shown in the previous session. Repeated non-attempts over four sessions with no apparent cause resulted in that subject being excluded from further sessions and this result recorded.

During sample placement, the tester and assistant ensured that the last jar touched was not the jar containing the TP and endeavoured to randomly touch more than two jars contained in the scent wheel prior to each run. A run was repeated five times in one trial with each placement of the TP being in a randomly allocated position. Once a trial was completed, the subject was rested prior to the next trial.

I recorded the number of attempts made by the individual subjects to perform a correct match. The number of correct first attempt matches divided by the total number of runs for that session was referred to as the success rate, e.g. if eight of the subject’s first attempts in 10 runs (two trials each of five runs) resulted in a correct match on that first attempt, then that subject was deemed to have an 80% success rate in that session. This was always calculated per level in a session.

2.3.4 Proxy Scenting

Human-scented proxies were scented by the scent donor either by the donor rubbing the proxy between their hands for 10 – 15 minutes or keeping the proxy inside their clothing with

maximum skin contact for the same period, as per Settle *et al* (1994), Schoon (1996) and Jezierski *et al* (2010). I did not control for soap or perfume odours on the donor as this was unnecessary for the objectives of this study. There was no published protocol for scenting of proxies with a non-human scent donor or a by-product of a scent donor. I therefore followed a similar protocol for human-scented proxies and draped the proxy over the scent donor or scent donor by-product (e.g. scat) for a similar period as that used with human-scented proxies, i.e. 10 – 15 minutes (Settle *et al*, 1994; Schoon, 1996; Jezierski *et al*, 2010). Some of the donor by-products had discernibly higher moisture content than other donor by-products. It was not possible to remove moisture from these "wet" samples, so to control for the different moisture content in by-product samples the by-products which were deemed "dry" (determined by whether any moisture was easily visually discernible) were sprayed with a single burst of tap water from a handheld spray bottle prior to the proxy being placed on the by-product. This procedure was followed each time proxies were scented with donor by-products. All donor by-products were stored in sealed jars when not in use for scenting proxies and the same by-products were utilised for the duration of this study.

Once scented, proxies were immediately individually stored in a clean transparent glass jar and labelled for future use with donor and scent transfer details noted. In many instances, these scented proxies were duplicated, i.e. the scent donor/by-product scented one proxy as a reference proxy and one proxy as a target proxy. Both proxies were always scented at the same time. In all other instances, the scented proxy was used as a decoy and not for matching, so a duplicate scented proxy was not required.

In this study a scented proxy was considered "fresh" if scent transfer onto the proxy had occurred less than 12 hours previous to the proxy being used in trials. If scent transfer onto the proxy was more than 12 hours before use in a trial, the proxy was considered "aged". Scent transferred to a secondary item, such as a proxy, is known to degrade over time (Schoon, 2005) making olfactory sampling more challenging to the dog. Therefore, only fresh proxies were used at the start of training levels. Once subjects consistently demonstrated a success rate of more than 80% in a particular level, aged proxies were occasionally used. The target scent was never changed during a trial, but was sometimes changed between trials within a single session.

2.3.5 Indication Behaviours

Studies using scent-matching dogs rely on the subjects to perform an "indication behaviour" or "alert", which is a clearly distinguishable behaviour illustrating the subject's choice of the target sample amongst the various samples available. Indication behaviours vary greatly with: sample retrieval (Kalmus, 1951; Schoon & De Bruin, 1994; Settle *et al*, 1994; Schoon, 1996); lying down or sitting adjacent to the sample (Kerley & Salkina, 2007; Wasser *et al*, 2009; Jezierski *et al*, 2010); staring/nose pointing at the sample (Fischer-Tenhagen *et al*, 2011); barking at the sample (Oesterhelweg *et al*, 2008) and scratching/pawing at the sample (Settle *et al*, 1994; Oesterhelweg *et al*, 2008). Studies generally state that the indication behaviour is determined by subject preference.

Typically, the indication behaviour (e.g. sitting or lying down) is regarded as being of great importance in demonstrating the subject's choice (Settle *et al*, 1994; Kerley & Salkina, 2007; Wasser *et al*, 2009; Jezierski *et al*, 2010; Fischer-Tenhagen *et al*, 2011). However, I elected to make the indication behaviour secondary to the interest shown in the sample by the subject. For example, if a subject had been trained to sit next to the chosen sample match, but on a particular training day the subject did not sit next to the sample but performed some other behaviour, which clearly showed interest – such as pawing at the sample – I did not consider this as a negative response. The reasons for this were twofold. Firstly, from a learning perspective it was more important to reinforce the subject's association with the target scent, i.e. success at matching, than to insist on an arbitrary behaviour which occurred after the desired behaviour had already been performed. While training of indication behaviour is important in order to provide the subject with a means to alert the handler its choice, it is secondary to the actual behaviour that the subject is being trained to perform, i.e. scent-matching. Insisting on the trained indication behaviour (such as sitting) for every single matching incidence may ultimately be counter-productive to the goals of the training. This point of view is supported by Fischer-Tenhagen *et al* (2011). In essence, the subject is required to perform a task (scent-match) and this should take priority over the complementary trained indication behaviour, with the absolute requirement that the subject's choice of sample (illustrated by an alternate behaviour of pawing, scratching etc.) is clear. Secondly, allowing the subjects to indicate in a manner in which they are most comfortable took ambient environmental factors in account, e.g. on a particularly hot day it may have been more comfortable for a subject to indicate by sitting instead of demonstrating the trained indication of lying down. From my own experience being flexible with the indication

behaviour appeared to allow the subjects to perform the task for a longer period, with greater accuracy and enthusiasm.

Allowing the subjects to vary indication behaviour could however not compromise results in anyway, so the onus of "making a call" as to the indicated sample rested on the handler, who was blind to the location of the target sample. A record of the initial trained indication behaviour (sitting, lying down) and any other indication behaviours displayed by subjects during training was kept.

In summary, an indication was deemed as the subject stopping at a holder and then making eye contact with the handler for at least two seconds, or the subject stopping at a holder and focusing its attention on this holder (sitting, lying down, staring, pawing or barking) for at least two seconds. The latter was required because in some instances the subject had its back to the handler and eye contact was not possible. In almost all instances, the subjects performed the trained indication behaviour (sitting or lying down) and in the other instances the indication of the sample was clearly shown by pawing or barking at the holder.

2.3.6 Training protocol

Scent-matching is a complex task and as such my training protocol was broken down into several levels of training. More levels of training allowed for smaller increments of learning between these levels, with the assumption being that multiple smaller increments of cognitive demand would increase the probability of end task success when compared to fewer larger increments of cognitive demand. Table 5 provides the detail of how the training of the end task (scent matching via proxy among species) was broken down into several levels.

Table 5. Levels of scent-match training via proxy by domestic dogs *Canis lupus familiaris* indicating the main objectives, modality use, absence/presence of decoys and target scent donor.

<table>
<tr><th>Level</th><th>Main Objective</th><th>Modality Use</th><th>Decoys</th><th>Target Scent Donor</th></tr>
<tr><td>Pre-Training: Level -2</td><td>Build a positive association to the proxy and encourage the subject to gain possession of the proxy through play</td><td rowspan="4">Bimodal (visual and olfactory)</td><td rowspan="4">Absent</td><td rowspan="6">Known (handler)</td></tr>
<tr><td>Pre-Training: Level -1</td><td>The subject gains possession of the proxy/locates the proxy from a distance</td></tr>
<tr><td>Pre-Training: Level -0</td><td>The subject substitutes an indication behaviour for proxy possession/location</td></tr>
<tr><td>Level 1 (L1)</td><td rowspan="6">Subject to locate and indicate proxy</td></tr>
<tr><td>Level 2 (L2)</td><td rowspan="2">Unimodal (olfactory)</td><td>Blank</td></tr>
<tr><td>Level 3 (L3)</td><td>Scented</td></tr>
<tr><td>Level 4 (L4)</td><td>Bimodal (visual and olfactory)</td><td>Absent</td><td rowspan="3">Unknown (variety of scent donor species)</td></tr>
<tr><td>Level 5 (L5)</td><td rowspan="2">Unimodal (olfactory)</td><td>Blank</td></tr>
<tr><td>Level 6 (L6)</td><td>Scented</td></tr>
</table>

The three pre-training levels aimed to create a positive association towards a proxy by the subject, through the use of play. In these pre-training levels, the subject was gradually required to use olfaction to locate the proxy as the tester hid the proxy. Once the subject was willingly locating the proxy, the proxy was contained in the scent wheel from that stage forward.

In all the pre-training levels and the next three levels (L1 – L3), the scent used as the target scent was a scent well known to the subject, i.e. the subject's handler, because it was assumed that due to the familiarity of the handler's scent, the task at hand would thus be less demanding of the subject. In L1, all six holders of the scent wheel contained jars, but the five decoy jars were empty – this allowed the subject to use a bimodal compound stimulus (visual and olfactory) to locate the proxy. This increased the likelihood of subject success. In L2,

each decoy jar contained a blank decoy and the target jar contained the handler scented proxy. In this level the subject was required to discriminate a scented decoy (known handler scent) from a non-scented (blank) decoy using a unimodal stimulus (olfaction). The next level, L3, was the same as L2, except that the decoy proxies were then scented, i.e. the subjects were required to not only discriminate a scented proxy, but a proxy of a particular scent, i.e. the handler. In the following three levels, L4 – L6, the same procedure was followed as L1 – L3 (no decoys, blank decoys, scented decoys) but with the target scent now unknown to the dog.

2.3.7 Training Level Promotion

In each training level subjects were required to meet certain criteria to pass to the next level of training within a minimum and maximum number of sessions (Table 6). In all Pre-Training levels, promotion criteria were relaxed (no statistical measures were used and competence was subjectively assessed by the tester). In L1 through L6, level promotion was non-subjective and determined statistically. A subject was required to achieve a statistically significant first attempt match rate (within a lower and upper session limit) prior to progressing to the next level of training. In view of the increasing level of difficulty as the training levels progressed, the minimum and maximum sessions allowed to achieve the promotion criteria were increased accordingly. Due to varying subject participation, i.e. an unequal number of runs between subjects during levels, it was not possible to have a set number of first attempt matches as a goal for level promotion, as used by Jezierski *et al* (2010) where 50 faultless trials over 100 attempts was used as promotion criteria between stages of training and testing. In my study, each subject's performance in each level was thus individually analysed.

Table 6. Level of training, promotion criteria, minimum sessions, maximum sessions, number of subjects promoted to next level and data recorded in training of scent-matching via proxy among species by domestic dogs *Canis lupus familiaris.*

<table>
<tr><th>Level</th><th>Promotion Criteria</th><th>Minimum Sessions</th><th>Maximum Sessions</th><th>Number of Subjects</th><th>Data Recorded</th></tr>
<tr><td>Pre-Training: Level -2</td><td>Subject showed interest in the target proxy as soon as it was produced</td><td>2</td><td>4</td><td>15</td><td rowspan="3">Competence achieved/not achieved</td></tr>
<tr><td>Pre-Training: Level -1</td><td>Subject located a partially obscured target proxy from about six metres</td><td>2</td><td>4</td><td>15</td></tr>
<tr><td>Pre-Training: Level -0</td><td>Subject indicated target proxy contained within target jar through pre-trained response</td><td>2</td><td>4</td><td>15</td></tr>
<tr><td>Level 1 (L1)</td><td rowspan="6">Subject indicated the random target proxy holder number in the scent wheel on first attempt at a statistically significant level ($p < 0.05$) across all sessions for that level. Scent donor and presence/absence of unscented/scented decoys accounted for the differences in L1 – L6</td><td>2</td><td>4</td><td>15</td><td rowspan="3">Target proxy holder number, number of attempts required for the subject to perform a match by indicating this holder.</td></tr>
<tr><td>Level 2 (L2)</td><td>2</td><td>4</td><td>15</td></tr>
<tr><td>Level 3 (L3)</td><td>2</td><td>4</td><td>13</td></tr>
<tr><td>Level 4 (L4)</td><td>2</td><td>4</td><td>13</td><td rowspan="3">As per L0 – L3 as well as scent donor, duration of proxy scenting, time of proxy storage</td></tr>
<tr><td>Level 5 (L5)</td><td>4</td><td>8</td><td>13</td></tr>
<tr><td>Level 6 (L6)</td><td>4</td><td>8</td><td>12</td></tr>
</table>

Only when all subjects in a training group had achieved a statistically significant success rate for that level was that group promoted to the next training level (the rationale for group treatment with regard to promotion criteria is described under Handler Management, below).

In no instances did subjects start a new level at the beginning of a session, i.e. new levels were always introduced during a session. Once subjects had been promoted to a higher level this did not mean that lower levels were never repeated, e.g. during L6 training subjects would often be given a "priming" L5 trial at the start of the session to ensure that target

proxies were scented and that the subjects were capable of scent matching at that given session, as suggested by Schoon (1996). In two separate instances two subjects (ML and CR) performed very poorly in a priming trial as described and their sessions were aborted. Both subjects were reported to be ill shortly thereafter (ML ear infection and CR gastroenteritis), which is similar to the findings reported by Settle *et al* (1994) where poor performance was either related to a break in training or the onset of illness.

2.3.8 Training Procedure

A training run, as described in the following section, was repeated five times for each subject within a trial and a trial was performed two to three times per subject per session, i.e. a subject performed a maximum of 15 runs within a session at any one level during early levels of training. If the subject showed reduced interest in the TJ, that subject's training session was immediately terminated and the subject recommenced training on this level at the following session.

Pre-Training: Level -2

Handler scent was transferred to a proxy (as described in Materials) prior to each training session, i.e. all of the handlers had scented their proxies immediately before a training session started. This handler-scented proxy was the target proxy (TP) for each subject for this and the following five levels of training.

Once the proxy was scented by the handler, the handler engaged the subject in off-lead play and encouraged the subject to mouth the TP. This served both to condition the subject to associate the TP with a pleasant activity (play) and also to engage the subject's scenting ability (in the dog, taste is thought to be closely associated with scent; (Fogle, 1990)). These play bouts were kept short to approximately 30 seconds each and were repeated a maximum of three times with an interval of approximately 60 seconds. Between play bouts, the TP was returned to the scenting position (either held by the handler or placed within their clothing making contact with bare skin). If the subject maintained interest throughout, the following level of training commenced in the same session. If the subject showed no interest or started losing interest in the TP, as determined by the subject averting its gaze from the TP, the session was terminated and this level was repeated at the next session.

Pre-Training: Level -1

The handler engaged the subject in off-lead play with the TP and then handed the TP to the tester, while lightly restraining the subject to build its enthusiasm. The tester continued to encourage the subject to play with the TP by waving and shaking it and moved about six metres away while the subject was still showing interest in the TP. The tester then animatedly placed the TP on the ground by waving their arms; the animated placement was required to maintain interest in the TP from the subject and to give a clear indication as to the location of the TP. The tester then left the test area quickly while the subject was focussed on the TP. On cue from the tester, the handler directed the subject to "Match/Find It" and then released the subject. As the subject approached the TP, the handler remained in the same position, i.e. away from the subject, but continued to verbally praise and encourage the subject. Once the subject had found the TP, the handler moved toward the subject rapidly and rewarded the subject with a food/toy item and verbal praise at the TP location.

The first run within a trial proceeded as described above and in each run thereafter the tester placed the TP in a partially concealed location (e.g. at the foot of a small table). If the subject returned to the handler with the TP, no attempt was made to take the TP away from the subject. In this instance the handler verbally praised the subject for at least five seconds, then requested the subject to give the TP up to the handler and rewarded the subject with a food or toy item. The TP was then either handed to the tester by the handler or in the case of a non-retrieve the tester picked up the TP off the ground. This process of finding the TP was repeated five times (runs) in that trial with an interval of approximately 30 seconds. At the end of a trial, the TP was returned to the scenting position and the subject was rested while the next subject underwent the same procedure. If the subject maintained interest in the TP throughout, the next level of training commenced in the same session. If the subject showed no interest or started losing interest in the TP, the session was terminated and this level was repeated at the next session.

Pre-Training: Level 0

The handler produced the TP and encouraged the subject to play with the TP without allowing the subject to gain possession of the TP. Ensuring that the subject was watching, the tester produced a transparent glass jar and the handler placed the TP into this jar, which was then referred to as the target jar (TJ). While the handler lightly restrained the subject, the tester moved approximately six metres from the subject and handler, and placed the TJ on the

ground where it could be easily seen by the subject; a very sturdy transparent glass container was used and these sessions were conducted outdoor on a grassed surface. The tester then immediately vacated the test area while the handler continued to restrain the subject. If the subject was showing interest in the TJ, the subject was released and cued to "Match/Find It".

Upon arriving at the TJ, the subject was not able to gain possession of the TP (as it had previously) because the TP was within the TJ. It was at this stage that the handler, who remained stationary throughout, gave the subject the cue to sit or lie down – this was the start of training an indication behaviour in the subjects. As soon as the subject correctly responded with the command to sit or lie down, the handler moved rapidly towards the subject and presented the subject with a food or toy reward, taking care to dispense either from as close as possible to the TJ (to associate the TP with reward) without actually touching the TJ (to avoid scent contamination of the TJ). This process of finding the TJ was repeated five times (i.e. five runs) in that trial with an interval of approximately 30 seconds. After the first successful run, the tester placed the TJ in a partially concealed location for each subsequent run (e.g. at the foot of a table). At the end of these trials, the TP was removed from the TJ, returned to the scenting position and the subject was rested while the next subject underwent the same procedure with its handler-scented TP, which was placed into a clean jar.

If the subject located the TJ but did not adopt the requested indication behaviour position (i.e. sit or lie down) after three such requests, the handler moved towards the subject and repeated the request with a verbal cue and a hand signal, if required. The subject was then rewarded in this position. In subsequent sessions, the cue from the handler for the indication (sit or down) was delayed, ideally to the point that the subject performed this indication without prompting from the handler. If a subject lost interest in the TJ, the session was immediately terminated and the subject repeated this level at the following session.

If a subject pawed excessively at the TJ, retrieved the TJ or managed to extract the TP from the TJ, the subject was not reprimanded and instead measures were taken to prevent this from occurring in the future i.e. the TJ could have been secured via a "u-shaped" metal stake into the ground, or the TP could have been placed deeper into the jar. None of the subjects retrieved the TJ, so the staking of the TJ was not required. Some of the subjects extracted the TP from the TJ and this was addressed by placing the TP deeper into the jar. It was critically important to not reduce the subject's enthusiasm for the task and even if these problems

persisted, the following level of training rectified these issues by preventing jar or proxy retrieval due to the design of the scent wheel. The problems mentioned were not of major concern to the training, whereas a reprimand at this early stage of training could have been severely counter-productive to the ultimate training goal as it could have reduced the subject's motivation to locate the proxy and possibly even created an aversive association towards a stimulus present at the time of reprimand, e.g. tester, proxy, jar.

If a subject failed to locate the TJ, the handler verbally encouraged the subject to find the TJ and, if needed, the tester physically encouraged the subject as well, by moving towards the TJ and encouraging the subject to do same. It was critically important that the handler remained away from the TJ because the subject had to learn that this task was performed independent of guidance from the handler. In my experience as an animal trainer, I have found that third party facilitation during the acquisition of a task that a dog is ultimately required to perform independent of their handler results in greater eventual success than when the same facilitation is performed by the handler.

Once a subject moved without hesitation to the TJ as soon as it was permitted to do so, the subject was introduced to the "scent wheel" (Figure 5). The same procedure was followed as described above, with the TJ being randomly placed in a different holder on each run instead of at the foot of the table. Once the subject had made a correct indication the handler moved towards the subject and delivered the reward as close as possible to the TJ taking care not to touch the holder, the TJ or the scent wheel for the reasons previously mentioned.

Training: Level 1

Up until this stage, the subject had been required to find the TP contained within the TJ, which was the only jar available. It was not known whether the subject was actually performing this behaviour or simply finding the only available jar. Level 1 training controlled for this possibility by training the subject to discriminate, i.e. not all jars were equal – some contained a target proxy (and were hence target jars) and some jars did not contain a proxy. This was accomplished by all holders containing a jar, but only one jar containing a TP (in this instance handler-scented), so there was only one TJ. Due to the increased cognitive demand of this level, the subject was aided in the process by having a bimodal compound stimulus (visual and olfactory) made available to it. This was accomplished through the transparent design of the scent wheel (Figure 5) where options were visually obvious and the

use of transparent glass jars made empty jars visually different to the TJ, which contained the TP. These aids assisted the subject in the process of decision-making and maximised the possibility of success while ensuring that the scent-association occurred.

Until this stage, the TP had been scented with a scent well known to the subject, i.e. the handler, which was assumed to be an established olfactory memory in the subject and hence not requiring sampling by the subject at the start of a run in order for the TP to be located. In later levels of training, scents unknown to the subject would be used to scent the TP. The subject would thus not have had an established olfactory memory of the scent on the TP. The subject would accordingly require a sample of the scent on the TP at the start of a run in order to establish a reference for the scent to be matched. The sample presented to the subject at the start of the run was the reference proxy (RP), for which there was always an identical TP in the line-up of samples presented to the subject. The subjects required training to sample the RP prior to a run being started. At this level of training, the concept of "giving scent" – where the handler presented a RP to the subject at the start of a run for olfactory sampling – and "taking scent" – where the subject sampled the RP via olfaction – was introduced. Introducing the RP process earlier in the training could possibly have created a stimulus overload on the subjects and waiting to introduce the concept later in training could have meant that opportunities to create the pre-run ritual of giving/taking scent would have been lost.

To train the procedure of giving and taking scent, the handler was now required to simultaneously scent two proxies prior to a session – one being the RP and the other being the TP. The handler held the RP lightly by a corner and allowed the subject to smell the RP or the handler lightly draped the RP over the subject's nose and/or muzzle. The subject was not forced to take scent from the RP, as this may have created an aversion to the proxy, which would have hampered the training progress. No subject was prevented from placing its mouth on the RP, which appeared to heighten the enthusiasm of some of the subjects. While the subject was taking scent, the handler gave the cue "Match/Find It" and once the handler was satisfied that the subject had taken scent from the RP (determined by the subject sniffing or mouthing the RP) the subject was released to perform the match to the TP contained in the scent wheel.

As in Level 0, the tester placed the TP into a jar (TJ) and then placed the TJ into a random holder in the scent wheel and five empty jars into the other holders. The subject took scent as described and was cued by the handler to "Match/Find It". The handler then remained silent and stationary until the subject demonstrated a clear choice at a holder on the scent wheel, either via the trained indication behaviour or another means of clear indication such as sitting, lying down, pawing, scratching or barking. The handler then called out the corresponding holder number to the tester. If the subject had correctly indicated the TJ, the tester called "Yes" and the subject was then rewarded as per Level 0. The subject then returned to the start position with the handler while the tester placed the TJ into another randomly allocated holder. I was not concerned that the subject watched the tester placing the TJ at this level because the jar holders were close enough together to ensure that the subject could not rely on an exact positional tester cue alone, i.e. the subjects did not appear to be able to spatially memorise the exact location of the TJ from the distance they were from the scent wheel at the start of a run.

In situations where the subject made an incorrect indication, the tester called "No" and the handler encouraged the subject to try again, without moving closer to the subject. If the subject failed to find the TJ after three indications, the subject was called back to the handler, took scent from the RP again and re-attempted to locate the TJ (re-cast). If a subject re-casted in one trial for more than three runs without success in finding the TJ, that trial was aborted for that subject and the next subject commenced its trial. If a subject experienced three aborted trials in a session, the subject returned to the previous level of training until competency was demonstrated, at which stage the subject re-attempted Level 1.

Training: Level 2

The procedure for this level was similar to the previous level, but now all jars contained a proxy – five of which were decoys (unscented proxies) and one was the handler-scented TP. The subject could now no longer rely on visual information to discriminate between jars. It was at this level that the subject had to rely on a unimodal stimulus (olfaction) to locate the randomly placed TJ among the unscented proxy decoy jars. Subjects also now commenced training in one of the other two training locations, i.e. subjects were no longer working on a grassed area.

Training: Level 3

The procedure for this level was similar to the previous level, but now all jars contained a scented proxy – only one of which was the handler-scented TP. The scented decoys were scented on humans both known and unknown to the dog, different species unknown to the dog and by-products (e.g. scat of various species, shed snake skin). This level differed from the previous level in that the subject was matching a scent on a reference proxy to the same scent on another randomly placed proxy and not just finding any scented proxy

Training: Level 4

The procedure for this level was similar to Level 1 in that there was only one randomly placed proxy for sampling (the TP) and all other jars were empty. However, at this level the scent on this proxy was foreign to the subject, compared to Level 1 where the scent on the proxy was well known to the subject, i.e. handler scent. The scenting of the foreign-scented proxy was the same as described in Level 3.

Training: Level 5

All jars contained a proxy – five of which were decoys (unscented proxies) and one was the randomly placed foreign-scented TP.

Training: Level 6

All jars contained a scented proxy – only one of which was the randomly placed target (foreign-scented proxy). The scented decoys were scented as described in Level 3.

2.4 Testing Runs

Each subject underwent a minimum of 80 L6 training runs spread over a minimum of seven L6 sessions. If no correlation between experience and successive L6 session success rate was present, i.e. a subject's success rate neither improved nor declined significantly between early and later L6 sessions, then these first 80 L6 runs for each subject were deemed testing runs. If a correlation was found between experience and successive session success rate in L6 training sessions, then an additional 80 runs on L6 were conducted and these were deemed testing runs. The data gained from these testing runs as described were used to establish proficiency at scent-matching among donor species via proxy by trained dogs.

2.5 Computer Simulated Dog

In order to test the null hypothesis, an untrained computer simulated dog (Digi Dog) was created. To generate the results of the Digi Dog the following procedure was followed. A table with eight columns was created. The first column was a listing of the run number, which ran from Run 1 to Run 80 (Table 7). The second column was populated with a randomly generated holder number (range 1 to 6), containing the simulated match. This process was repeated for 80 runs. The third column (first attempt) was populated with another series of 80 randomly generated numbers (range 1 to 6). This procedure was repeated for the remainder of the columns (second, third, fourth, fifth and sixth attempt), thereby creating a series of six holder number choices per run by the simulated untrained dog. In the ultimate null model, the randomly generated numbers in columns three to eight, i.e. the simulated choices, would always comprise the full range of choices, namely 1 to 6. However in the interests of simulating an actual run it was decided to only use random numbers within the range which had not appeared before. This was done because a handler would not allow a repeat incorrect indication and a match would thus always occur even if purely by a process of elimination. Digi Dog was thus guaranteed to make a match in six attempts, with the only variable being how many attempts were required to make that match. When the number in the second column (randomly generated holder number) was the same as the number in an attempt column, this attempt cell was highlighted. The total number of highlighted cells per attempt column were then totalled for the 80 runs. This process was replicated to create the same number of Digi Dogs as actual subjects, i.e. 12 times.

Table 7. Example of computer-simulated dog indicating random holder numbers over five scent-matching runs during one trial, detailing run number, random target holder number, holder numbers indicated randomly by computer-simulated dog, attempt on which a match was made between a reference and a target sample and total numbers of attempts required to perform a match with shaded areas indicating a match.

Run Number	Random Target Holder Number	Random Holder Number Indicated By Computer-Simulated Dog					
		First Attempt	Second Attempt	Third Attempt	Fourth Attempt	Fifth Attempt	Sixth Attempt
1	3	2	1	3	4	5	6
2	4	1	6	5	3	4	2
3	6	5	1	4	6	2	3
4	5	5	6	1	4	3	2
5	1	4	2	3	1	6	5
Totals		1	0	1	2	1	0

In the abbreviated example of five runs provided (Table 7), the Digi Dog scored: 1 first attempt match; 0 second attempt matches; 1 third attempt match; 2 fourth attempts matches; 1 fifth attempt match and 0 sixth attempt matches.

2.6 Removing Bias

When an animal is required to perform a trained response to a stimulus presented to the animal, there is a high likelihood of unintentional cues being provided to the animal by the trainer or handler, which could thus bias the animal's response (Pfungst, 1911). In this instance, while it appears that the animal is responding correctly, the animal is in fact merely picking up external cues. A classic example of this problem is the case of Clever Hans, a horse which was thought to be able to perform simple arithmetic via hoof taps, but was later found to be following subconscious body movement cues from the trainer and/or audience (Pfungst, 1911). It was thus imperative that great care was taken to ensure that the subjects in this project were scent-matching the donor species and not using unintentionally-provided cues to make a decision as to which sample matched the reference sample. This section covers areas of potential bias and details how these were dealt with.

All jars and the scent wheel were only ever touched by the tester or assistant and always while wearing powder-free latex gloves. The tester or assistant placed jars in the scent wheel with the handler and dog out of sight – either in another room or with their backs to the scent wheel. The tester called the handler and dog into the training area only once all jars had been placed and the tester or assistant had moved away from the scent wheel to a location which remained the same in every run. During jar placement the tester and assistant always ensured that last jar touched was never the TJ.

In L2, L3, L5 and L6 of training, the runs were single-blind, i.e. neither the handler nor the subject were aware of the location of the TJ, but the tester and assistant were aware of the TJ location. The tester and assistant therefore avoided unintentionally providing a location cue to the handler or subject by avoiding eye contact with both prior to each run commencing and remaining so until the run was complete. In L1 and L4 of training the handler was aware of the TJ location, as no other proxies were present apart from the TP. The handler was however instructed to remain silent and allow the subject to perform the "match to sample" task independently as if all of the other holders contained proxies.

Each jar in a line-up (L1 – L6) had an identical label irrespective of contents. The content of the jar was handwritten on all labels at the start of a trial, prior to the proxy being inserted into the jar – this ensured that there was no non-proxy indicator of the target, such as wetter ink on the label. Similarly, care was taken to ensure that all labelling used a similar number of letters and hence a similar amount of ink appeared on each label to further reduce any non-proxy indicator and hence bias. In instances where the decoy jars were empty (L1 and L4) this was written on the label of the empty jars. Writing on the label was not visible to the handlers due to their distance from the jars.

Due to the "caged" design of the jar holders on the scent-wheel, subjects were unable to make any physical contact with the TJ or TP, and thereby possibly "mark" the TJ or TP with saliva for subsequent runs. It was however impossible to know whether subjects left any type of scent residue on the TJ or TP merely by being in close proximity to the TJ or TP. It would have been impractical to control for this variable by replacing every jar and proxy after every single run for each subject – in a normal group training session, this would equate to 450 jars and proxies being utilised (five subjects each performing 15 runs, each comprising six jars and proxies). However, this factor was investigated by comparing the performance of

subjects in the first run in a session, when all jars and proxies were new compared to their performance in subsequent runs when the jars and proxies were not new. If the subjects were found to have a significantly lower success rates in new proxy first runs when compared to subsequent runs, scent residue on the TJ or TP could have been introducing a bias. Initial data comparisons did, however, indicate a significantly different success rate between new proxy first runs and chance.

The handling of all proxies (target, reference, scented and unscented decoys) during a session was identical with the tester and assistant using metal tongs while wearing powder-free latex gloves and the handlers using powder-free latex gloves.

The TP was randomly allocated to one of the six holders in the scent wheel. Random holder repetitions were incorporated to ensure that a holder was never discounted either by the handler or the subject as a potential match because it was a match in the prior run, i.e. there were always six options available because prior runs did not influence future placement.

2.7 Handler Management

Settle *et al* (1994) noted that the performance of scent-matching dogs declined when the handler of the dog became emotionally invested in the success of the subject. In this study it was impossible to control for this by keeping the subject's performance unavailable to the handler because the handler verbalised the subject's choice of holder and was then immediately informed by the tester whether this was a match or a non-match, i.e. the handler was an essential component of the scent-matching process. I planned to address this potential problem by making handlers aware that emotional investment in the subject's performance could hinder performance and I thus briefed handlers prior to the start of training to remain as emotionally detached as possible.

Handlers were also advised that subjects would only be promoted to the next training level once all the subjects in a training group had demonstrated a good understanding of the current level. This was done so as not to alienate any one handler in a group, e.g. if a single subject in a group performed poorly it could have been embarrassing to that handler for their dog to remain on a lower level while the rest of the group was promoted. This embarrassment could have resulted in reluctance by the handler to attend future sessions and hence the loss of a subject from the study after a considerable amount of time had already been invested. The

drawback to this approach was that handlers could have felt pressured if their dog was performing poorly and holding the rest of the group back from promotion to the next level. Handlers may well have felt this self-inflicted pressure, but it did not appear to impact negatively on the subject's performance. This was possibly due to the fact that handlers were briefed to remain as emotionally detached as possible as noted previously.

2.8 Data Analysis

Per level analysis of first attempt matches for each subject were required to determine if promotion criteria to the next training level had been met and this was analysed as follows. On each first attempt in any run, the subject had the option of one of six samples and the likelihood of random success was thus 16.667%. If a subject was observed to perform a first attempt match this was scored as follows: 100% (observed success) and 16.667% (expected success). If a subject was not observed to make a first attempt match, this was scored as follows: 0% (observed success) and 16.67% (expected success). Each subject's runs in a level were analysed in this manner using a chi-square test.

Prior to any statistical analysis being conducted on L6 test runs, it was necessary to establish whether these runs could be grouped and analysed as a set or whether a statistically significant change in L6 success rates (number of first attempt matches divided by the total number of L6 runs in that session) over time needed to be considered. Data for the L6 sessions were compared over time for each subject using Spearman rank correlation.

It was then necessary to establish whether the subjects were placing a mark on the unknown target proxy, i.e. on the first run with a new target proxy were the first number of match attempts random until the target proxy was found and then the target proxy somehow marked by the subject for identification on future runs? New proxy first runs were analysed for L5 and L6 because in many instances the new proxy was first used in L5 runs and then in L6 runs in the same session. This test was not used to measure scent-matching, but rather "new proxy marking". In order to test for the possibility for later identification, all new proxy first runs for L5 and L6 were extracted from the data for all subjects. On each first attempt in any of these runs, the subject had the option of one of six samples and the likelihood of random success was thus 16.667% (one of six). A first attempt match by the subject was scored 100% and all other attempt matches were scored 0%. This was compared to an expected match of 16.67% using chi-square test for all new proxy first runs in L5 and L6.

To assess whether dogs are capable of scent-matching via proxy among donor species, I then compared the observed likelihood of random success at scent-matching with the expected success at scent-matching by each subject for each of the subject's 80 L6 testing runs. The same procedure was used as detailed above, i.e. first attempt match was scored 100%, all other attempt matches were scored 0% and this was compared to an expected match of 16.67% using chi-square test.

To investigate whether a dog trained to scent-match via proxy among donor species would have a higher success rate than an untrained computer-simulated dog, each subject's number of attempts to perform a match over the 80 test runs were listed. The same process was followed for 12 Digi Dogs. The subject's data was then compared to each Digi Dog's data using a Generalized Linear Model with ordinal multinomial distribution and a logit link function. The dependant variable was attempt number, the categorical predictor was subject name and the count variable was matches made (Table 8).

Table 8. Example of the number of attempts required to perform a match across 80 scent-matching runs by a trained domestic dog *Canis lupus familiaris* and an untrained computer-simulated dog (Digi Dog).

Subject Name	Attempt Number	Matches Made
ZN	1 (First)	56
	2 (Second)	14
	3 (Third)	7
	4 (Fourth)	3
	5 (Fifth)	0
	6 (Sixth)	0
Digi Dog 1	1 (First)	20
	2 (Second)	8
	3 (Third)	11
	4 (Fourth)	14
	5 (Fifth)	16
	6 (Sixth)	11

Covariates such as breed, proxy scenting method and donor species were analysed using chi-square test where the expected number of first attempt matches (obtained by the mean number of first attempt matches observed across the categories of the covariate being examined) was compared to the actual number of first attempt matches in the 80 test runs with respect to the covariate in question. Variables such as age, sex, training/testing venue and training group were analysed by recording the number of attempts required to perform a match (depending on the variable being examined) and then comparing each potential predictor against each other potential predictor within that category (e.g. males *vs.* females) by using a Generalized Linear Model with ordinal multinomial distribution and a logit link function. The dependant variable was attempt number, the categorical predictor was the subset of age, sex, training/testing venue or training group, and the count variable was matches made.

Chapter 3: Results

3.1 General

The 12 subjects which completed training and testing underwent a total of 4 083 runs, of which 1 153 were L6 runs (Table 9).

Table 9. Total training sessions and runs across training levels during scent-match training of domestic dogs *Canis lupus familiaris.*

Subject	Training Group	Sessions	L1	L2	L3	L4	L5	L6	Total
ZR	1	33	28	92	38	13	194	157	522
NM			25	55	24	16	45	101	266
FF			15	58	20	20	119	130	362
ML			24	57	26	18	123	109	357
SC	2	30	31	108	29	30	76	87	361
EM			31	93	29	21	77	83	334
CH			31	75	29	22	59	80	296
HP			31	98	39	18	76	80	342
BB	3	30	31	88	34	18	68	80	319
PC			23	78	27	18	55	84	285
CR			28	86	27	13	74	82	310
VC			31	95	34	18	71	80	329
Totals		**90**	**329**	**983**	**356**	**225**	**1037**	**1153**	**4083**

3.2 Level Promotion

Table 10 lists the number of runs completed prior to promotion out of a level, as well as the first attempt matches and the probability of this being a chance event. Runs completed prior to promotion ranged from: 15 – 31 (L1); 10 – 68 (L2); 10 – 39 (L3); 10 – 30 (L4) and 23 – 49 (L5). Total runs within a level before the criteria for promotion out of that level was achieved were: 261 (L1); 536 (L2); 308 (L3); 208 (L4) and 494 (L6).

Table 10. Subject name, number of sessions, runs, first attempt matches (%), chi-square value and probability of random match as criteria out of training levels in the training of domestic dogs *Canis lupus familiaris* to scent-match a scented proxy in the presence of five scented decoys.

Subject	L1					L2					L3					L4					L5				
	Sessions	Runs	FAM (%)	χ^2	P value	Sessions	Runs	FAM (%)	χ^2	P value	Sessions	Runs	FAM (%)	χ^2	P value	Sessions	Runs	FAM (%)	χ^2	P value	Sessions	Runs	FAM (%)	χ^2	P value
ZR	2	20	60	51.2		3	51	56	120.18		3	29	93	112.52		2	10	100	41.55		5	49	73	151.75	
NM	2	15	90	58.34		2	10	90	37.56		2	10	90	37.56		2	13	100	54.02		5	23	67	63.66	
FF	2	15	85	46.37		2	11	100	45.87		2	11	62	25.6		2	15	80	50.36		7	45	63	119.18	
ML	2	15	75	54.35		2	12	90	45.71		2	10	80	37.73		2	12	73	37.9		7	49	63	131.81	
SC	3	31	75	96.9		5	60	87	213.41		4	29	81	96.57		3	30	53	68.82		6	43	96	170.69	
EM	3	31	95	124.82		5	60	80	201.45		4	29	84	100.56		3	21	38	35.41		6	49	64	131.81	
CH	3	31	100	128.81	< 0.001	4	45	100	186.98	< 0.001	4	29	80	96.57	< 0.01	3	22	54	51.53	< 0.02	5	35	58	85.61	< 0.001
HP	3	31	91	116.84		5	60	79	197.46		5	39	91	150.09		2	18	49	38.9		6	48	64	131.64	
BB	3	31	96	124.82		5	58	63	153.26		5	34	70	101.39		2	18	83	62.83		5	43	68	122.83	
PC	2	23	94	92.91		6	68	88	246.65		4	27	100	112.19		2	18	83	62.83		4	28	84	100.39	
CR	3	31	70	92.91		5	51	69	148.1		4	27	84	96.23		2	13	72	38.06		5	41	72	122.5	
VC	3	31	80	104.88		5	50	74	151.92		5	34	76	109.37		2	18	95	70.8		5	41	58	106.56	

Session and run totals detailed above did not equal the overall number of sessions as the figures in Table 10 were the minimum sessions and runs required to meet promotion criteria. Runs are also not always divisible by five (normal number of runs within a trial) because on some occasions a trial with less than five runs was conducted for the reasons mentioned in the Methods (Training: Sessions). The large ranges in number of runs between subjects achieving promotion criteria and the number of total runs between levels were due to two reasons. Firstly, individual subjects did not on frequent occasions progress to the next training level despite having met criteria because the entire training group was required to meet these criteria prior to the group being promoted to the next training level. Secondly, new levels were only started during a training session (never at the beginning of a session), so if one subject member of a group could not attend the session at which a new level was to be introduced, then the subjects remained on the current level until the next session when the whole group was present at which time the whole group was promoted to the next level. In these instances the run results were included in the promotion criteria data, provided the maximum session criteria had not been exceeded.

As a group, the dogs achieved level promotion success rates ranging from 69.03% to 84.36%, with a range of 305 – 536 runs required to realise promotion to the next training level (Table 11).

Table 11. Training levels in scent-matching a proxy in the presence of five scented decoys among species by a group of domestic dogs *Canis lupus familiaris* detailing the average percentage of success, number of runs within each level and level ratio of success to runs prior to promotion.

Training Level	Average Success Rate	Number Of Runs	Level Ratio (success/runs)
L1	84.36%	305	3.62
L2	81.37%	536	6.59
L3	82.61%	308	3.73
L4	73.25%	208	2.84
L5	69.03	494	7.16

The average success rates per level are expressed as a ratio.

3.3 L6 Session Success Rates Over Time

No statistically significant correlation between L6 session success rate and time was found for any of the subjects ($p < 0.05$) (Table 12). Only 1 subject (FF) displayed a relatively high negative R value (-0.628), which was below a significant level and this was the only instance among the group of subjects. Therefore, the first 80 runs in L6 per subject were grouped and used for each subject to assess proficiency at scent-matching via proxy.

Table 12. Success rate percentage per Level 6 session and Spearman rank correlation values between success rates over sessions during Level 6 scent-match training in domestic dogs *Canis lupus familiaris.*

Subject	L6 Session Success Rate (%)														R	p
	L6 Session Number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
ZR	20	50	60	60	95	100	40	50	88	66	80	80	84	93	0.496	> 0.05
NM	100	60	85	66	73	9	93	-	-	-	-	-	-	-	-0.214	
FF	93	80	100	65	66	60	60	74	70	-	-	-	-	-	-0.628	
ML	100	92	40	60	80	73	80	0	90	38	50	90	-	-	-0.326	
SC	80	0	20	88	30	60	60	70	80	90	-	-	-	-	0.463	
EM	60	20	63	20	40	40	40	60	20	-	-	-	-	-	-0.182	
CH	14	0	88	0	100	60	10	100	66	-	-	-	-	-	0.412	
HP	25	0	100	60	70	60	37	80	72	-	-	-	-	-	0.485	
BB	40	40	80	30	70	60	50	50	-	-	-	-	-	-	0.289	
PC	90	77	20	90	80	90	90	66	100	-	-	-	-	-	0.313	
CR	100	25	20	90	60	0	50	90	80	100	-	-	-	-	0.207	
VC	100	0	80	50	100	50	70	80	83	-	-	-	-	-	0.076	

3.4 New Proxy First Run Success Rates

A total of 65 new proxy first runs were conducted in L5 and L6, among 11 subjects (subject SC was never used first in a training session simply due to the random allocation of subject order in the sessions). Of these 65 new proxy first runs, first attempt matches occurred in 37 instances, second attempt matches in 12 instances, third attempt matches in 12 instances, fourth attempt matches in 4 instances and no instances of fifth or sixth attempts were recorded. This was significantly different to the match attempts that would have occurred by chance ($\chi^2 = 158.412$, $df = 64$, $p < 0.001$) indicating that the subjects were not matching new target proxies randomly.

3.5 Subject Success Rates

At an individual level, first attempt matches by the subjects ranged from 41.25% (Subject EM) to 78.75% (Subject PC). All subjects that completed the training demonstrated first attempt matches at a level statistically greater than chance, which was 16.67% on each first attempt (Table 13).

Table 13. Subjects, first attempts correct, success rate, comparison to expected chance success and probability of random success in scent-matching a proxy in the presence of five scented decoy proxies by trained domestic dogs *Canis lupus familiaris*. n= 80, df = 79

Subject	Level 6 runs		χ^2	p
	First attempts correct	Success rate		
ZR	56	70%	237.27	< 0.001
NM	62	77.5%	261.26	
FF	58	72.5%	385.24	
ML	61	76.25%	257.26	
SC	49	61.25%	209.28	
EM	33	41.25%	145.30	
CH	44	55%	189.28	
HP	47	58.75%	201.28	
BB	44	55%	189.28	
PC	63	78.75%	265.26	
CR	61	76.25%	257.26	
VC	57	71.25%	241.27	

3.6 Subject Success Rates Compared To Untrained Dogs

In the 80 L6 test runs, instances among the subjects of first attempt matches were 634 (range 33 - 63) (Table 14). Second attempt matches were 185 (range 6 – 28); third attempt matches were 113 (range 2 – 29); fourth attempt matches were 22 (range 0 – 6) and fifth attempt matches were observed in six instances (range 0 – 1). None of the subjects required a sixth attempt to scent match the reference proxy to the target proxy in the 80 L6 test runs.

Table 14. Attempts required by 12 trained domestic dogs *Canis lupus familiaris* to scent-match a proxy in the presence of five scented decoy proxies over 80 runs per subject detailing subject name, total attempts required, proportion of match attempts and standard errors.

Subject	Attempts required for match					
	First	Second	Third	Fourth	Fifth	Sixth
ZR	56	14	7	3	0	0
NM	62	10	5	3	0	0
FF	58	13	8	0	1	0
ML	62	13	2	2	1	0
SC	49	13	11	6	1	0
EM	33	28	13	5	1	0
CH	44	6	29	0	1	0
HP	48	27	5	0	0	0
BB	42	21	14	2	1	0
PC	63	12	5	0	0	0
CR	60	15	4	1	0	0
VC	57	13	10	0	0	0
Totals	634	185	113	22	6	0
Proportion of attempts made to obtain a match (%)	66.04	19.27	11.77	2.29	0.63	0
SE	2.76	1.90	2.08	0.60	0.15	0

The Digi Dog data to perform a match of the hypothetical reference proxy to the hypothetical target proxy are presented in Table 15.

Table 15. Attempts required by 12 computer simulated dogs (Digi Dog 1 - 12) to hypothetically scent-match a proxy in the presence of five scented decoy proxies over 80 runs per subject. Total attempts required, proportion of match attempts and standard errors are reported.

Subject	Attempts required to match					
	First	Second	Third	Fourth	Fifth	Sixth
DD1	20	8	11	14	16	11
DD2	18	12	10	15	12	13
DD3	18	10	16	6	16	14
DD4	16	4	15	18	14	13
DD5	9	15	13	15	13	15
DD6	12	18	19	7	11	13
DD7	11	16	12	15	10	16
DD8	8	12	14	17	11	18
DD9	15	17	14	12	12	10
DD10	18	17	10	11	15	9
DD11	14	13	8	15	12	18
DD12	12	14	16	14	12	12
Totals	171	156	158	159	154	162
Proportion of attempts made to obtain a match (%)	17.81	16.25	16.46	16.56	16.04	16.88
SE	1.12	1.19	0.90	1.06	0.58	0.83

When trained subjects were individually compared to each of the 12 Digi Dogs, the subjects showed a difference from the Digi Dogs with regards to the number of attempts required to match a scented reference proxy to a target proxy (Table 16). The subjects displayed a pattern of decreasing multiple attempts and the Digi Dogs displayed a random spread of attempts.

Table 16. Comparison of 12 trained domestic dogs *Canis lupus familiaris* and 12 computer-simulated dogs with regard to the number of attempts (maximum 6) required to match a scented proxy in the presence of five decoys proxies over 80 runs. Wald χ^2 statistic scores are presented at df = 1, p < 0.01 for shaded comparisons, p < 0.001 for unshaded comparisons.

Subject	Computer Simulated Dogs											
	DD1	DD2	DD3	DD4	DD5	DD6	DD7	DD8	DD9	DD10	DD11	DD12
ZR	47.13	48.16	48.65	58.83	64.58	54.19	59.55	69.22	49.47	44.16	56.86	58.06
NM	51.88	53.44	53.85	62.45	69.47	60.46	64.91	73.34	55.62	50.35	61.74	63.41
FF	49.71	50.89	51.44	61.63	67.34	57.48	62.44	71.86	52.57	46.97	59.41	61.14
ML	53.17	52.02	55.85	63.99	71.29	63.19	66.94	74.93	57.94	52.26	63.27	65.69
SC	35.86	36.20	36.51	46.90	51.40	39.55	46.21	56.57	35.80	31.64	45.05	44.23
EM	27.84	27.50	23.18	40.96	43.48	30.04	37.55	50.00	26.29	21.90	36.71	35.90
CH	31.80	31.14	30.41	44.23	44.83	30.79	39.51	51.04	28.53	24.87	39.89	37.57
HP	47.09	47.87	49.13	61.36	65.07	54.86	59.90	70.19	49.50	43.05	56.76	59.25
BB	34.91	34.89	35.34	48.18	51.20	38.24	45.44	57.36	34.22	29.34	44.04	43.88
PC	56.23	57.90	58.50	67.55	73.71	65.58	69.47	77.33	60.57	54.78	65.97	68.42
CR	53.86	55.39	56.17	65.64	71.57	63.00	67.13	75.49	57.88	52.00	63.71	66.09
VC	49.80	50.74	51.08	62.12	67.10	56.65	62.08	71.82	51.97	46.40	59.33	60.80

As a group, the trained subjects performed a successful match, as follows: on first attempt 66.04% (n = 634, SE = 2.76); second attempt 19.27% (n = 185, SE = 1.90); third attempt 11.77% (SE = 2.08); fourth attempt 2.29% (n = 22, SE = 0.60) and fifth attempt 0.63% (n = 6, SE = 0.15). None of the trained subjects required a sixth attempt to perform a match. When grouped, the 12 Digi Dogs performed a successful match on: first attempt 17.81% (n = 171, SE = 1.12); second attempt 16.25% (n = 156, SE = 1.19); third attempt 16.46% (n = 158, SE = 0.90); fourth attempt 16.56% (n = 159, SE = 1.06); fifth attempt 16.04% (n = 154, SE = 0.58) and sixth attempt 16.88% (n = 162, SE = 0.83).

3.7 Age, Sex, Breed, Reproductive Status Effects

The age of the dog at the start of training was rounded to the nearest whole year. Six age classes were identified (Table 17) with the majority of the ages falling within two to six years, with a lower limit of one year and an upper limit of eight years. The subjects aged less than three years and older than five years had a higher first attempt match rate than the subjects aged three and five years.

Table 17. Age of subjects, number of subjects and attempts required to perform a match (presented in number and proportion) in scent-matching a proxy in the presence of five scented proxy decoys over 80 runs per subject by trained domestic dogs *Canis lupus familiaris.*

Age of subject at start of training	n	Attempts required to match (Percentage of attempts required to match)					
		First	Second	Third	Fourth	Fifth	Sixth
1 year	1	57 (71.25%)	13 (16.25%)	10 (12.5%)	0 (0%)	0 (0%)	0 (0%)
2 years	4	227 (70.94%)	68 (21.25%)	21 (6.56%)	4 (1.25%)	0 (0%)	0 (0%)
3 years	1	42 (52.5%)	21 (26.25%)	14 (17.5%)	2 (2.5%)	1 (1.25%)	0 (0%)
5 years	4	184 (57.5%)	60 (18.75%)	61 (19.06%)	11 (3.44%)	4 (1.25%)	0 (0%)
6 years	1	62 (77.5%)	13 (16.25%)	2 (2.5%)	2 (2.5%)	1 (1.25%)	0 (0%)
8 years	1	62 (77.5%)	10 (12.5%)	5 (6.25%)	3 (3.75%)	0 (0%)	0 (0%)

When ages groups were compared to each other, two clusters emerged, i.e. no difference existed between subjects aged three and five years (the two ages groups with the lowest success rate), but dissimilarity was found with these two age groups and the remaining age groups (one, two, six and eight years old), with the youngest and oldest dogs being similar to one another in terms of having the highest success rates (Table 18).

Table 18. Age of subjects and comparison of number of attempts required to match a proxy in the presence of five scented proxy decoys over 80 runs per subject by trained domestic dogs *Canis lupus familiaris.* Wald χ^2 statistic scores are presented at df = 1, p < 0.05 for shaded comparisons, p > 0.05 for unshaded comparisons.

Age of subject at start of training	Attempts required to match (Percentage of attempts required to match)					
	1 year	2 years	3 years	5 years	6 years	8 years
1 year	-	0.03	5.93	5.91	0.95	0.67
2 years	-	-	12.68	19.12	1.25	0.91
3 years	-	-	-	0.16	11.24	9.96
5 years	-	-	-	-	11.50	10.57
6 years	-	-	-	-	-	0.01

The sexes were equally represented in the 12 subjects which were tested in matching a scented proxy in the presence of five scented decoy proxies. Males achieved a 68.96% first attempt match rate and females 63.33% (Table 19). There was no significant difference between males and females in respect of numbers of attempts required prior to a match being indicated (Wald χ^2 statistic score = 1.396, df = 1, p = 0.237).

Table 19. Sex of subjects, number of subjects and attempts required to perform a match (presented in number and proportion) in scent-matching a proxy in the presence of five scented proxy decoys over 80 runs per subject by trained domestic dogs *Canis lupus familiaris.*

Sex of subject	n	Attempts required to match (Percentage of attempts required to match)					
		First	Second	Third	Fourth	Fifth	Sixth
Males	6	328 (68.33%)	77 (16.04%)	67 (13.96%)	6 (1.25%)	2 (0.42%)	0 (0%)
Females	6	306 (63.75%)	108 (22.5%)	46 (9.58%)	16 (3.33%)	4 (0.83%)	0 (0%)

Due to the low number and broad diversity of breeds represented in the test group, subjects were clustered into one of the ten breed groups of dogs as defined by the Fédération Cynologique Internationale (Table 20). In the instances of the four cross-breed subjects (FF,

BB, CR and VC) the subjects were assessed by three suitably qualified canine specialists (Shannon McKay, Belinda Thomas, Adrienne Hawkins) and then assigned to a breed group with which they were deemed to have the most morphological similarities.

Table 20. Categorisation of domestic dog *Canis lupus familiaris* subjects into breed groups, number of subjects and success rates in matching a scented proxy in the presence of five scented decoys in 80 runs.

Breed Group	n	Success Rate
Sheepdogs and Cattle Dogs	4	69.06%
Pinschers and Schnauzers	1	70.00%
Dachshunds	1	72.50%
Retrievers, Flushing Dogs and Water Dogs	6	62.29%

Breed was not a significant predictor of first attempt matches ($\chi^2 = 0.008$, df = 3, p = 0.999).

From L2 forward two training and testing venues were utilised – one indoor and one outdoor. Subjects completed all post L2 training and testing at one of these two venues, i.e. no subject alternated venues during training or testing. Four subjects (Group 1) completed training and testing indoors and recorded a combined first attempt match rate of 74.1% (Table 21). Eight subjects (Group 2 and 3) completed training and testing outdoors and recorded a combined first attempt match rate of 62.5%.

Table 21. Training venue of subjects, number of subjects and attempts required to perform a match (presented in number and proportion) in scent-matching a proxy in the presence of five scented proxy decoys over 80 runs per subject by trained domestic dogs *Canis lupus familiaris.*

Training venue of subject	n	Attempts required to match (Percentage of attempts required to match)					
		First	Second	Third	Fourth	Fifth	Sixth
Indoor	4	238 (74.38%)	50 (15.63%)	22 (6.88%)	8 (2.5%)	2 (0.63%)	0 (0%)
Outdoor	8	396 (61.88%)	135 (21.09%)	91 (14.22%)	14 (2.19%)	4 (0.63%)	0 (0%)

When subjects were grouped into training venue, i.e. indoors vs. outdoors, the subjects trained and tested indoors showed a significantly higher first attempt match rate than the subjects trained and tested outdoors (Wald χ^2 statistic score = 14.842, df = 1, $p < 0.001$).

Each subject group comprised four subjects during testing. Combined first attempt match rates amongst the groups were as follows: Group 1 (74.1%); Group 2 (54.7%) and Group 3 (70.3%) (Table 22).

Table 22. Training group of subjects, number of subjects and attempts required to perform a match (presented in number and proportion) in scent-matching a proxy in the presence of five scented proxy decoys over 80 runs per subject by trained domestic dogs *Canis lupus familiaris.*

Training group of subject	n	Attempts required to match (Percentage of attempts required to match)					
		First	Second	Third	Fourth	Fifth	Sixth
Group 1	4	238 (74.38%)	50 (15.63%)	22 (6.88%)	8 (2.5%)	2 (0.63%)	0 (0%)
Group 2	4	174 (54.38%)	74 (23.13%)	58 (18.13%)	11 (3.44%)	3 (0.94%)	0 (0%)
Group 3	4	222 (69.38%)	61 (19.06%)	33 (10.31%)	3 (0.94%)	1 (0.31%)	0 (0%)

A significant difference existed between Group 1 and Group 2 (Wald χ^2 statistic score = 28.580, df = 1, $p < 0.001$), with regard to the number of attempts required to perform a match, with Group 1 performing better than Group 2. Group 2 also differed significantly from Group 3 (Wald χ^2 statistic score = 17.924, df = 1, $p > 0.001$) with Group 3 performing matches in fewer attempts. There was no difference in the number of attempts required to perform a match between Group 1 and Group 3 (Wald χ^2 statistic score = 1.677, df = 1, $p = 0.20$).

3.8 Proxy Scenting Method, Donor Species Effects

All proxies were either scented via body contact with the scent donor or via contact with a scent donor by-product, i.e. scat, dung or shed skin of the donor (Table 23). Of the 960 testing runs conducted, 270 used body scented proxies for the target scent and 690 used by-product scented proxies for the target scent. With body scented proxies, subjects demonstrated a first attempt match rate of 72.59% and with by-product scented proxies a first attempt match rate of 63.48% was observed. Scent donors for the proxies (both targets and decoys) comprised 15 species, including mammals, reptiles and birds (Table 23). First attempt match rates ranged from 89.83% (Corn snake *Pantherophis guttatus guttatus*) to 40% (ring-necked parakeet *Psittacula krameri*).

Table 23. Listing of scent donor species in descending first attempt match rate, number of runs conducted, proxy scenting method and first attempt match rate in scent matching of a scented proxy in the presence of five scented decoys among species by 12 trained domestic dogs *Canis lupus familiaris*.

Scent donor species	Number of runs	Proxy scenting method (contact)			First attempt match rate
		Body	By product		
			Scat / Dung	Shed skin	
Corn snake *Pantherophis guttatus guttatus*	59	X			89.83%
Elephant *Loxodonta Africana*	92		X		85.87%
Aurora house snake *Lamprophis aurora*	41	X			85.37%
Puff adder *Bitis arietans*	46			X	82.61%
Human *Homo sapiens*	58	X			72.41%
Angolan free-tailed bat *Mops condylurus*	20	X			70%
House snake *Boaedon capensis*	21	X			66.67%
Cheetah *Acinonyx jubatus*	134		X		64.18%
Hippo *Hippopotamus amphibius*	112		X		58.93%
African buffalo *Syncerus caffer*	146		X		58.9%
Night adder *Causus rhombeatus*	30	X			56.67%
Burchell's zebra *Equus quagga burchellii*	105		X		55.24%
File snake *Mehelya capensis*	36	X			52.78%
Common duiker *Sylvica pragrimmia*	55		X		45.46%
Ring-necked parakeet *Psittacula krameri*	5		X		40%

Whether the proxy was scented on the donor's body or a by-product of the donor had no effect on the subject's first attempt match rate ($\chi^2 = 0.006$, df = 1, p = 0.937). The species of the scent donor did not influence the first match attempt rate of the subjects ($\chi^2 = 1.337$, df = 14, p = 0.999).

Chapter 4: Discussion and Conclusions

4.1 General

My study aimed to establish whether the domestic dog *Canis lupus familiaris* is able to discriminate between a scent sample from a donor amongst a group of samples from donors of other species, with all samples being scented proxies. I used an expanded training programme (nine levels) and unique device (a scent wheel). I predicted that trained dogs would be able to match a scented proxy with a success rate significantly different to chance ($p < 0.05$), and that when compared to an untrained computer simulated dog, trained dogs would have a significantly higher success rate ($p < 0.05$). I found that domestic dogs can be trained to discriminate a scent sample via a proxy from a donor presented among five scent samples from donors of other species, with a success rate significantly greater that chance. When compared to untrained computer-simulated dogs, the trained dogs achieved a significantly higher success rate in matching a scented proxy.

While my study was unique, because I used scent donors from multiple species, it is worthwhile to compare the overall success rates of this study with recent single-species donor studies. In Settle *et al* (1994) a success rate of 83% was recorded and in Jezierski *et al* (2010) a success rate of 58%.I obtained an overall success rate of 66%, much lower than that of Settle *et al* (1994), which could be due to an increased level of difficulty in my study; in the Settle *et al* (1994) study the dogs did not need to process species-specific olfactory information as all target and decoys scents were from one known species (humans). In my study, the dogs were required to both process species specific olfactory information and were exposed to novel species scent. Kerley & Salkina (2007) note that novelty in target and decoy scents appear to improve the performance of scent-matching dogs. This could account for the slightly higher success rate in my study when compared to Jezierski *et al* (2010). In my study, dogs were frequently exposed to novel target and decoy scents whereas Jezierski *et al* (2010) used only scents from people. It is thus possible that while increasing the novelty of scents presented to the dogs may increase the level of difficulty for the dogs, this novelty could also increase the dog's engagement in the task (evidenced by higher success rates). Success rates in scent-matching training programmes could perhaps be a vector for exploring the interaction between novelty and challenge in olfactory sampling tasks.

4.2 Training Accounts

Accounts of olfactory training in dogs are scarce (Schoon & De Bruin, 1994; Schoon, 2005; Jezierski 2010; Hall *et al*, 2013). I kept records of 4 082 training runs across all levels of training, of which 960 were Level 6 testing runs to contribute to canine olfactory training accounts.

Training was split into multiple levels (three Pre-Training and six Training levels) leading up to the testing runs. This decomposition of a complex task is in keeping with the notion that in order to acquire a complex task, the task needs to be broken down into small increments (Krueger & Dayan, 2009) with the level of difficulty gradually increasing as the training approximates closer to the end goal. When analysing the level performance per subject, I expected a general trend of success rates decreasing as the training level increased, due to the increased difficulty. This trend was seen with an exception of L2 and L3, where L3 success rates (82.61%) were slightly higher than L2 success rates (81.37%). This difference was quite small and in all other instances success rates were negatively correlated with level number, i.e. with each subsequent level that the dogs completed the success rate became lower. The only other study which reports on progress during training levels of scent-matching in dogs (Jezierski *et al*, 2010) noted a similar trend.

However, when the number of runs was taken into account and a "level score" (the average level success rate/number of runs prior to promotion) was calculated some unexpected results were found. L2 and L5 level scores (6.59 and 7.16 respectively) were very dissimilar to the other level scores, which ranged between 2.84 (L4) and 3.62 (L1). L2 and L5 contained blank decoys, which was believed to be an easier task for the dogs to discern than L3 and L6 (where all decoys were scented). This unexpected disparity is possibly due to the "unperceptive perceiver" problem, i.e. humans cannot perceive the discriminating olfactory information provided to the dogs, so I made an educated guess as to the degrees of difficulty. I assumed that once decoys were added, that blank decoys would be easier to discern than scented decoys. However, it appeared that the blank decoys were just as difficult for the dogs to discriminate as a scented decoy. Because humans cannot conduct olfactory sampling at the same level as dogs, it is very possible that even the blank decoys contained scent that needed to be sampled and analysed by the dogs, making L2 and L5 (unscented decoys) just as difficult as L3 and L6 (scented decoys). It is also likely that the dogs expected to find scent on these unscented decoys and when no scent was present this somehow disturbed the learning

process. The “unperceptive perceiver” problem experienced in this study was a good reminder that the properties of a stimulus can vary depending on the perceptive abilities of the observer (Auffarth, 2013). Future studies on canine olfaction skills should bear this in mind.

Multiple levels of training could also possibly provide early indicators of eventual success as noted by Jezierski *et al* (2010) and thus save time by removing poorly performing dogs earlier in the training. I expected that fairly consistent patterns would emerge with regard to the individual’s performance per level, i.e. a poor performer in lower levels would exhibit poor performance at the higher levels. However, this trend did not emerge (e.g. EM and CH scored the highest levels of success in L1 of training and the lowest levels of success in L6). I did not find any early indicators of success in scent matching, as also observed by Jezierksi *et al* (2010). Why no early predictors of eventual success could be identified could be due to multiple reasons, including training programmes that have been constructed with inappropriate measures of difficulty (unperceptive perceiver problem) or a learning phenomenon peculiar to dogs. My study was not aimed at establishing if and how early predictors of scent-matching success exist, but this is an interesting topic which deserves further study.

4.3 Modality Use

This study differed from previous studies in that subjects had the use of a compound bimodal stimulus (visual and olfactory) during multiple stages of training. In studies of scent-matching within species via proxy where the modality available to the subject during training was unimodal (olfaction), success rates varied from 58% (Jezierski *et al,* 2010) to 83% (Settle *et al*, 1994). In my study, the overall success rate was 66%. The results from these studies are not directly comparable because my study considered scent-matching among species whereas Settle *et al* (1994) and Jezierski *et al* (2010) addressed scent-matching within a species. While bimodal stimulus use in training of scent-matching did temporarily slow the learning process, it did not appear to hinder the subjects in this study with regard to overall scent-matching success. It is interesting to note that bimodal stimulus (visual and olfactory) was also used in very early training stages of the programme that has the highest recorded success rate of 83% (Settle *et al*, 1994).

4.4 Sample Arrangement & Distance

A unique device (scent wheel) was used in this study, which allowed for samples to be placed closer together than previously done in the literature and also in a circular pattern as recommended by Wasser *et al* (2009). While the scent-matching success rates of the subjects in this study were high, without exclusion studies, it is not possible to state that the success rates were due to one particular factor, such as the scent wheel. However, it can be concluded that compared to the success rates of similar training programmes without a scent wheel, the new device did not appear to negatively impact success rates of scent-matching via proxy of several scent donor species.

The circular design did present one problem. When the subject was unsure of a sample choice, it almost always selected a sample closest to the handler, which allowed the subject to face the handler, i.e. it would work its way around the scent wheel and if a sample choice was not apparently obvious, the subject would indicate the sample second closest to the handler, excluding the closest sample (which would necessitate the subject not having easy eye contact with the handler). To avoid this problem in the future I would recommend that eye contact between the subject and the handler be made neither easier nor more difficult through the modified placement of the holders. This can be easily accomplished by retaining the scent wheel in its current form but with the holders in an arc instead of a complete circle, with the base of the arc perpendicular to the handler's position.

4.5 Subject Sample Size & Source

Previously, sample sizes in canine scenting studies have been small due to two main reasons – financial and ethical considerations in specifically acquired dogs (Kerley & Salkina, 2007; Richards *et al*, 2008; Wasser *et al*, 2009) and primary obligations of active police service dogs (Settle *et al*, 1994; Schoon & De Bruin, 1994; Schoon, 1996, 2005). In a few instances, pet dogs, also referred to as companion dogs (Brisbin & Austad, 1991; Fischer-Tenhagen *et al*, 2011) have been used in scent studies. In my study, companion dogs were used exclusively, which allowed for the largest sample (15 started, 12 completed) compared to all previous similar studies. Companion dogs appear to be capable of being trained to scent match by proxy for several donor species and present none of the ethical, monetary or logistical problems associated with specifically acquired and police service dogs, i.e. the subjects can live as companion dogs, no long term care is required, subjects are freely available and are mostly exempt from other obligations.

Subjects in this study were not selected with the intention of maximising the number of subjects capable of scent-matching, i.e. subjects were not "cherry picked". Of the 15 subjects that were recruited, 12 completed the training and testing which demonstrates that companion dogs are capable of learning complex olfactory tasks.

Settle *et al* (1994) reported that handlers became emotionally invested in their dog's success rates which appeared to impact negatively on the dog's success. Handlers in my study were thus briefed prior to the training commencing to remain as emotionally detached as possible. Despite this briefing, some handlers did become emotionally invested in their dog's success rates and this appeared to impact negatively on performance. Some handlers also appeared to become competitive with the other handlers. In future studies using companion dogs, it would be advisable to pay close attention to handler management to minimise detrimental emotional investment by the handlers and reduce competitive behaviour amongst handlers, both of which could have a negative influence on the subject's success rates.

4.6 Subject Variables

Some subjects performed better than others with success rates ranging from 41.3% to 78.8%. While a success rate of 41.3% appears to be very low, it must be remembered that this was a first attempt match when presented with five decoys, which would have been 16.7% by chance.

The variation in individual performance was expected for two reasons. Firstly, subjects were not selected based on any type of learning or olfactory ability. Secondly, multiple factors play a role in olfactory task performance, including motor functions, motivation, sensory abilities, learning and memory capacities (Kraemer & Apfelbach, 2004). In a canine learning study, it has been stated that behavioural variation is ever-present, which could partly be due to variations in personality (Wilsson & Sinn, 2012). Schoon & De Bruin (2004) noted variations in performances of scent-matching dogs and recommend regular examinations to verify performances over time.

The younger subjects (< 3 years of age) and the older subjects (> 5 years of age) were more proficient at scent-matching than the dogs between these age ranges. I did not expect to find subject age being predictive of success at scent-matching. Older rats show a decrease in

olfactory function (Mobley *et al* 2014, Morena *et al* 2014) and Mirich *et al* (2002) comment that change could be due to an increased threshold for olfactory perception in aged mice. In contrast, the oldest dogs in my study achieved the highest success rates overall. This is however not in conflict with the studies cited above for two reasons. Firstly, the oldest dog in my study was eight years old, which is not considered old for that particular medium-sized breed of dog (Border Collie). In Settle *et al* (1994), the lowest success rate (64%) occurred in the oldest subject (aged 10 years). Secondly, Kraemer & Apfelbach (2004) noted that while sensitivity to olfactory stimulus does decrease with age, there is no observable deficit in learning ability associated with olfactory tasks in rats. Therefore, it is possible that even if the older dogs did have a higher threshold for olfactory perception that the amount of scent transferred to the proxies exceeded this perception threshold. In other words, even if the older dogs had reduced olfactory sensitivity, there was sufficient scent on the proxies for the older dogs to perceive this information and achieve the high success rates observed.

The comparatively lower success rates of subjects aged between three and five years was unexpected. This is a difficult phenomenon to explore for two reasons. Firstly, there is paucity in the literature on canine cognition during middle-age with studies generally focussing on either very young or very old dogs. Secondly, the age group in question is not specific, i.e. the adult age range of dogs is poorly defined as being from approximately two years of age to 10 years of age. Studies on the effect of middle-age and learning in other species therefore cannot be applied because the oldest dogs in my study (> 6 years) would be included in the middle-age group. The dogs aged less than three years showed a high success rate similar to the older dogs, as also noted by Settle *et al* (1994).

The age effect was unexpected and with the limited sample size in my study it would require further investigation to establish whether this apparent relationship between age and success at scent-matching is causative or coincidental.

Sex of the subjects did not influence scent-matching success rates. This is to be expected due to the fact that scenting ability in an operantly-conditioned task, to a previously neutral scent, i.e. a scent not previously associated with gain or loss, would not be sexually dimorphic behaviour in domestic dogs. No other scent-matching study has either controlled for sex of the subjects or reported a difference in scenting ability between males and females (Kalmus, 1951; Brisbin & Austad, 1991; Schoon, 1996; Schoon & De Bruin, 1994; Settle *et al*, 1994;

Schoon, 2005). In some studies, the sex of the dogs used to scent-match is not even reported (Kerley, 2004; Wasser *et al*, 2008).

Breed type did not play a role in scent-matching success in my study. In support, other scent-matching studies did not report a breed influence on success rates (Kalmus, 1951; Brisbin & Austad, 1991; Schoon, 1996; Schoon & De Bruin, 1994; Settle *et al*, 1994; Schoon, 2005). These studies however did not explore whether a subject's breed was a predictor of scent-matching success and breeds selected were among the traditional working breeds (guarding, herding, gundogs) expected to perform well at operantly-conditioned olfactory tasks. The consistent exclusion of some breeds (e.g. toy breeds and sight hounds) from scent studies *per se* does however suggest that these breeds would not be expected to perform well at olfactory tasks. It cannot therefore be stated that breed is not a predictor of success at scent-matching, but rather that no difference exists amongst the breeds which have been selected to perform scent-matching studies to date.

A recent study on the proficiency of various breeds in a human-pointing direction task revealed that while breed was associated with differences in initial performance, with future training these differences were equalised across breeds (Udell *et al*, 2014). Although Udell *et al* (2014) report on a simple task (a dog following a human-pointing gesture) when compared to scent-matching by proxy, it is possible that while some breeds may be slower to acquire a task it is not unreasonable to expect that any breed of dog would be capable of scent-matching.

4.7 Training Variables

In my study, two venues were used from L2 training onwards – one indoor and one outdoor (comprising a floor, roof and one wall). The subjects trained indoors had a significantly greater success rate than the subjects trained outdoors. This was to be expected as scent-match training is typically performed indoors to limit the interference of weather conditions and distractions (Settle *et al*, 1994; Schoon & De Bruin, 1994; Schoon, 1996; Schoon, 2005; Kerley & Salkina, 2007; Wasser *et al*, 2009; Jezierski *et al*, 2010). However, when success rate was analysed per training group, there was no difference between the indoor group (Training Group 1) and one of the outdoor groups (Training Group 3). Whether scent-matching training occurs indoors or outdoors (as described above) does not appear to make a significant difference to eventual success rates at scent-matching via proxy among species. This is possibly due to ample scent transfer on the proxy and the dogs learning to discard

unnecessary information, such as visual/auditory distractions, from which the dog may be shielded in an indoor training venue. Considering the "unperceptive perceiver" problem (Auffarth, 2013) it is also possible that an indoor venue could be saturated with scent imperceptible to humans. This scent would more than likely not dissipate as freely as would occur in an outdoor venue. An indoor training venue could thus perhaps create an even more challenging environment for dogs to learn olfactory tasks.

Training Group 2 scored significantly lower on success rates than the other two groups. There are many reasons that could contribute to this result and it would be speculative to make a definitive claim as to why Training Group 2 performed poorly. I observed that two subjects (CH and SC) mouthed the group reference proxy excessively, rendering it very wet. It is possible that the excessive moisture and scent transferred on this proxy by these two subjects reduced the olfactory information available on this proxy – to all of the group subjects– and thereby increased the difficulty of the task, which could have reduced success rates for this group. Future studies should consider multiple reference proxies if possible to avoid this potential problem.

Proxies scented on the body of a scent donor were not associated with a higher success rate than proxies scented on a by-product of a scent donor. Proxy scenting on donor by-products has not been studied before, so there was no knowledge with regard to whether a difference would exist between skin-scented and by-product-scented proxies. Notwithstanding, three aspects must be considered prior to concluding that no difference exists between skin-scented and by-product-scented proxies. Firstly, skin-scented proxies were scented on "refreshed" donors, but by-product proxies were scented by repeatedly using the same by-product, i.e. the skin-scenting donors did not remain in the same state from one proxy scenting to the next proxy scenting (e.g. humans washed, animals shed skin cells) but the by-products remained in the same state. In order for a by-product sample to be "refreshed" in a manner similar to the skin-scenting donors, a new by-product sample from the same donor would have been required for each instance of by-product proxy scenting. The by-product samples were not "refreshed" in such a manner. Secondly, the bottled by-product sample could have degraded over time and thus less scent could have been available for transfer onto the proxy during proxy-scenting. No supporting evidence for this idea exists in the literature. Schoon (2005) mentioned that scented proxies have a presumed initial drop in amount of scent on the proxy, which stabilised over time, but this was a scented proxy and not the item used to scent the

proxy. Thirdly, it was possible that the by-product-scented proxies could have had a more complex scent profile (e.g. due to the combination of actual donor scent, food material, metabolic processes) than the skin-scented proxies, which could influence proxy scenting success rates. Any of the factors listed above could have had a positive or negative effect on the quality of the by-product for proxy scenting, but the design of this study was not sufficient to reveal any such influences. These factors and the interplay between these factors mentioned would need to be assessed prior to concluding that no difference exists between skin-scented and by-product-scented proxies with regard to the ability of dogs to scent-match these proxies.

Whether a trained dog would be able to match a skin-scented proxy from a donor with a by-product-scented proxy from the same donor was not tested in this study. However, as long as a sufficient amount of donor scent is present in the by-product, and this scent is suitably transferred onto a proxy, a dog should be able to match a donor skin-scented proxy to a donor by-product-scented proxy.

Species of scent donor were not associated with higher or lower success at scent-matching. This was expected because all dogs were naïve to the non-human donor species in this study, so no aversion or attraction to scent donor species was anticipated. It is however not possible to state that the donor species used in this study are all equal in terms of by-product proxy scent transfer quality. As stated above, all by-product-scented proxies were scented using the same sample repeatedly over time. The by-product sample used for scent transfer could have simply been a particularly good or particularly poor sample for scent transfer onto proxy and not necessarily a reflection of the quality of the species by-product for proxy-scenting. To investigate whether some species produce higher or lower quality by-products for proxy-scenting would require multiple samples from individuals of the same species collected at different times, ages and locations.

New proxies were detected at levels significantly different to chance. A comparison between new proxies and repeated use proxies was not performed because a “priming” effect (the dogs have had repeated exposure to the target scent with the repeated use proxies) would most likely occur, so any repeated proxy would be expected to be associated with higher success rates compared to a new proxy.

4.8 Conclusion and future studies

This studied aimed to assess whether domestic companion dogs are able to discriminate a scent sample from a donor among a set of scent samples from donors of other species (all via proxies) through the use of an expanded training programme and unique device. As predicted, the trained dogs were able to scent-match a proxy as described at levels significantly greater than chance, noting that in this study specificity and generalisation of this scent-matching was not tested. This study was worthwhile both in terms of testing a tool which could be of use in olfaction studies in the future and in terms of reducing the paucity of training accounts in scent training in dogs

In a broader perspective, this study highlights a number of topics. Firstly, the study has emphasised that olfaction is a multi-dimensional phenomenon with much that is unknown about both olfactory properties and perception of these properties among non-human species. As a relatively anosmic species it is important for us to accept that the perceptual properties of a stimulus are not defined according to our human perceptions, but are dependent on the perceptive abilities of the observer (Auffarth, 2013). In other words, just because we do not perceive a quality of a stimulus, does not mean that the quality does not exist. Secondly, we cannot know exactly what cognitive processes are being engaged by other animals, but by establishing a system whereby a choice (being a physical manifestation of decision) is clearly discernible, we are provided with a tangible outcome, which can perhaps be used to tease apart cognitive processes. Finally, this study has produced many proven and reliable olfaction testers at low cost, relative ease and in a fairly short period of time. The subjects trained in this study could be used in an olfaction study at any given moment and provide immediate, cheap and useful data to researchers for academic, commercial or civil purposes.

More studies need to be conducted to assess the limitations of scent matching via proxy by domestic dogs among species. The possibility of the dogs employing alternative behavioural/learning strategies during the training of scent-matching was not considered in this study and hence this remains an area that should receive further attention. This study showed that breed, sex and training venues do not need to be controlled for whereas age of the subjects might need to be considered. Reproductive status of the subjects could not be assessed in this study because all subjects which completed training were sterilised, so this is an aspect that should be regarded in future studies. Group training sessions and group promotion (the progression of the training group as a whole to the next level of training) could

impede individual progress and it is possible that better results could be obtained if dogs were to be trained individually. In addition, more levels of training also allowed for a finer measurement of the task acquisition process, i.e. a relationship between the stages of learning could possibly be established and not just a “before learning” and “after learning” set of data. Extracting finer data from the different levels of training, or increasing the levels of training even further, could highlight both early indicators of eventual success and levels of greater/lesser cognitive demand during training. Greater knowledge of these two aspects of scent-match training could lead to the development of a more effective scent-matching training protocol and this could be considered for future studies.

References

Auffarth, B. 2013. Understanding smell – The olfactory stimulus problem. *Neuroscience and Biobehavioral Reviews* **37:** 1667 – 1679

Brisbin I.L., Austad S.N. 1991. Testing the individual theory odour of canine olfaction. *Animal Behaviour* **42:** 63 – 69

Browne, C., Stafford, K., Fordham, R. 2006. The use of scent-detection dogs. *Irish Veterinary Journal Volume* **59(2):** 97 – 104

Davison, M.C., Tustin, R. D. 1978. The relation between the generalized matching law and signal-detection theory. *Journal of the Experimental Analysis of Behaviour* **29(2):** 331 – 336

Demant, H., Ladewig, J., Balsby, T.S.J., Dabelsteen, T. 2011. The effect of frequency and duration of training sessions on acquisition and long-term memory in dogs. *Applied Animal Behaviour Science* **133:** 228 – 234

Enquist, M., Ghirlandia, S. 2005. Neural networks and animal behaviour. Princeton University Press

Fischer-Tenhagen, C., Wetterholm, L., Tenhagen, B., Heuwieser, W. 2011. Training dogs on a scent platform for oestrus detection in cows. *Applied Animal Behaviour Science* **131:** 63–70

Fogle, B. 1990. The Dog's Mind – Understanding your dog's behaviour. Wiley Publishing Inc, Hoboken, New Jersey

Fukuhara, R., Tanaka, J., Yamaguchi, T., Ogura, G., Ukuta, H., Roy, S. 2010. Development and introduction of detection dogs in surveying for scats of small Indian mongoose as invasive alien species. *Journal of Veterinary Behaviour* **5:** 101 – 111

Gazit, I., Terkel, J. 2003a. Domination of olfaction over vision in explosives detection by dogs. *Applied Animal Behaviour Science* **82:** 65 – 73

Gazit, I., Terkel, J. 2003b. Explosives detection by sniffer dogs following strenuous physical activity. *Applied Animal Behaviour Sciences* **81:** 149 – 161

Hall, N.J., Smith, D.W., Wynne, C.D.L. 2013. Training domestic dogs (*Canis lupus familiaris*) on a novel discrete trials odour-detection task. *Learning and Motivation* **44(4):** 218 – 228

Jezierski, T., Swiergiel, A.H., Górecka-Bruzda, A., Walczak, M., Świergiel, A.H., Chruszczewski, M.H., Pearson, B.L. 2010. Operant conditioning of dogs *(Canis familiaris)* for identification of humans using scent lineup. *Animal Science Papers and Reports (*Institute of Genetics and Animal Breeding, Jastrzebiec, Poland **28(1):** 81 – 93

Kalmus, H., 1955. The discrimination by the nose of the dog of individual human odours and in particular of the odour of twins. *British Journal of Animal Behaviour* **3:** 25 – 31

Kehoe, E. J., Home, A, J., Home, P. S. Macrae, M. 1994. Summation and configuration between and within sensory modalities in classical conditioning of the rabbit. *Animal Leaming and Behavior* **22:** 19 – 26

Kerley, L. 2004. Scent Dog Monitoring of Amur Tiger (2003-0087-018). *Save The Tiger Fund*

Kerley, L. L., Salkina, G.L. 2007. Using scent-matching dogs to identify individual Amur tigers from scats. *Journal of Wildlife Management* **711:**1349 – 1356

Kirk, R.E. 1994. Experimental Design: Procedures for the behavioural sciences. Wadsworth Publishing

Kraemer, S., Apfelbach, R. 2004. Olfactory sensitivity, learning and cognition in young adult and aged male Wistar rats. *Physiology & Behavior* **81:** 435 – 442

Krueger, K., Dayan, P. 2009. Flexible shaping: How learning in small steps helps. *Cognition* **110:** 380 – 394

Meyer, I., Ladewig, J. 2008. The relationship between number of training sessions per week and learning in dogs. *Applied Animal Behaviour Science* **111:** 311 – 320

Mirich, J.M., Williams, N.C., Berlau, D.J., Brunjes, P.C. 2002. Comparative study of aging in the mouse olfactory bulb. *Journal of Comparative Neurology* **454:** 361 – 372

Mobley, A.S., Rodriguez-Gil, D.J., Imamura, F., Greer, C. A. 2014. Aging in the olfactory system. *Trends in Neurosciences* **37(2):** 77 – 84

Mondadori, C., Waser, P.G., Huston, J.P. 2005. Time-dependent effects of post-trial reinforcement, punishment or ECS on passive avoidance learning. *Physiological Behaviour* **18:** 1103 – 1109

Moreno, M., Richard, M., Landrein, B., Sacquet, J., Didier, A., Mandairon, N. 2014. Alteration of olfactory perceptual learning and its cellular basis in aged mice. *Neurobiology of Aging* **35:** 680 – 691

Oesterhelweg, L., Krober, S., Rottmann, K., Willhoft, J., Braun, C., Thies, N., Puschel, K., Silkenath, J., Gehl, A. 2008. Cadaver dogs—A study on detection of contaminated carpet squares. *Forensic Science International* **174:**35 – 39

Pfungst, O. 1911. Clever Hans (The horse of Mr. Von Osten): A contribution to experimental animal and human psychology. Henry Holt & Company

Richards, K.M., Cotton, S.J., Sandeman, R.M. 2008. The use of detector dogs in the diagnosis of nematode infections in sheep faeces. *Journal of Veterinary Behaviour* **3:** 25 – 31

Rowe, C. 2005. Multisensory learning: from experimental psychology to animal training. *Anthrozoös* **18(3):** 222 – 235

Schoon, G. A. A. 1996. Scent identification lineups by dogs (*Canis familiaris*): experimental design and forensic application. *Applied Animal Behaviour Science* **49:**257 – 276

Schoon, G. A. A. 2005. The effect of the ageing of crime scene objects on the results of scent identification line-ups using trained dogs. *Forensic Science International* **147:**43 – 47

Schoon, G.A.A., de Bruin, J.C. 1994. The ability of dogs to recognize and cross match human odours. *Forensic Science International* **69:**111 – 118

Settle, R. H., Sommerville, B. A., McCormick, J., Broom, D.M. 1994. Human scent-matching using specially trained dogs. *Animal Behaviour* **48:**1443–1448

Smith, D.A., Ralls, K., Maldonado, J.E., Hurt, A., Adams, B., Parker, M., Davenport, B., Smith, M.C. 2003. Detection and accuracy rates of dogs trained to find scats of San Joaquin kit foxes (*Vulpes macrotis mutica*) *Animal Conservation* **6:** 339 – 346

Sommerville, B.A., Settle, R.H., Darling, F.M.C., Broom, D.M. 1998. Short Communications – The use of trained dogs to discriminate human scent. *Applied Animal Behaviour Science* **57:**269 – 286

Szetei, V., Miklósi, Á., Topál, J., Csányi, V. 2003. When dogs seem to lose their nose: an investigation on the use of visual and olfactory cues incommunicative context between dog and owner. *Applied Animal Behaviour Science* **83:**141 – 152

Udell, M.A.R., Ewald, M., Dorey, N.R., Wynne, C.D.L. 2014. Exploring breed differences in dogs (Canis familiaris): does exaggeration or inhibition of predatory response predict performance on human-guided tasks? *Animal Behaviour* **89:** 99 – 105

Walczak, M., Jezierski, T., Górecka-Bruzda, A., Sobczyńska, M., Ensminger, J. 2012. Impact of individual training parameters and manner of taking breath odour samples on the reliability of canines as cancer screeners. *Journal of Veterinary Behaviour* **7:** 283 – 294

Wasser, S.K., Smith, H., Madden, L., Marks, N., Vynne, C. 2009. Scent-matching dogs determine number of unique individuals from scat. *Journal of Wildlife Management* **73(7):**1233 – 1240

Wilsson, E., Sinn, D. L. 2012. Are there differences between behavioral measurement methods? A comparison of the predictive validity of two ratings methods in a working dog programme. *Applied Animal Behaviour Science* **141:** 158 – 172