



Searching for the Greater Capricorn beetle

– A methodological review of training dogs
for a conservational purpose.

*På jakt efter större ekbock – en metodutvärdering för träning av
specialsökhundar i bevarandesyfte.*

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Swedish University of Agricultural Sciences, SLU
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Abstract

The Greater Capricorn beetle *Cerambyx cerdo* is an endangered species both in Sweden (CR) and internationally (VU). The substrate, old Oak, dropped heavily in numbers in Sweden during the 19th century, which left the beetle without suitable habitats.

The only remaining population is on Öland, an island on the Swedish east coast. At Nordens Ark, the species has been reared since 2012 and in the years of 2018, 2019 and 2020, they placed adults of the Greater Capricorn beetle on suitable trees on the Swedish mainland. This was done to re-establish the species where remnants had been found within the last 100 years. The result of the experiment has a five year delay due to the time it takes for the egg to become a beetle since the larva lives inside the wood for four years.

A study was designed in order to train two Irish softcoated wheaten terriers, Puma and Loka, to locate the Greater Capricorn beetle larva by scent. The aim was to monitor and evaluate this method.

The collected data is from training sessions held October 2019 to July 2021. This time period was divided into four slots during analysis of the data. The Accuracy was higher in the first period compared to the last for both dogs. Puma had a higher level of Sensitivity compared to Loka in period four.

Motivation wise, the number of reminders given by the handler, the number of times the dog lost its' focus and the number of times the dog asked for help had a positive correlation with the time in search.

Both dogs managed to locate the target scent with success, when within reach, and to differentiate the scent from other larva, in a controlled environment, but more work remains in order for the dogs to communicate when they find targets out of reach on oak trees.

Keywords: Dog search, Conservation detection dog, *Cerambyx cerdo*, *Quercus*, dog behaviour, Swedish oak history

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Abbreviations

CMR	Capture-Mark-Recapture
CDD	Concervation Detection Dog
IUCN	International Union for Concervation of Nature
SLU	Swedish University of Agricultural Sciences
VES	Visual Encounter Survey

1. Introduction

1.1. Background

1.1.1. The Greater Capricorn beetle

This species has been found in the southern parts of Sweden the last couple of hundred years but is today limited to only one locality on the island Öland on the east coast of Sweden in the Baltic sea.

Conservation

In Sweden there are two species of *Cerambyx*, *C. cerdo* (Linné, 1758) and *C. scopoli* (Fuessly, 1775). They are both endangered in Sweden but with different conservational status and different ecological requirements. *C. cerdo* is listed as critically endangered (CR) and *C. scopoli* as near threatend (NT) (Ljungberg et al., 2020). Internationally, only the Greater Capricorn beetle is considered threatened and is listed as vulnerable (VU) according to the IUCN Red List of Threatened Species (World Conservation Monitoring Centre, 1996). It is listed in both Annex II and IV of the Habitats Directive (92/43/EEC) which means that each EU-country has to form Natura-2000 areas to ensure that the species habitat is protected and it also requires a strict protection regime all across the species natural range (Carpaneto et al., 2017).

Morphology

The Greater Capricorn beetle is the largest species of longhorn beetles *Cerambycidae* in Scandinavia with its 40-53 mm (Bílý & Mehl, 1989). The adult is black with exception of the apical third of the elytra which is brown-red and it has long antennae reaching the elytral apex on the female while being twice the body length on males (Fig. 1). The body is robust, prolonged and the dorsal side has almost no hair in contrast to the ventral side which has fine yellow-brown hairs (Bílý & Mehl, 1989; Ehnström, 2007).

The larva is white and measures 80 mm long and 20 mm wide at the most and has two pair of legs, a yellow tained head and blackish jaws (Fig. 1; Ehnström, 2007).



Figure 1 On the left is a photo of the adult Greater Capricorn beetle taken by Claes Andrén and on the right is a photo of the larva taken by Jimmy Helgesson.

Swedish history

The Greater Capricorn beetle was first described by Linné in 1758 but it wasn't until in 1802 that Carl Fredrik Fallén, a professor at Lund University, made the first official Swedish find. The finding was made in the north east part of the province of Skåne, in the southern most part of Sweden. The next finding was made in 1827, on Öland, a Swedish island and province on the East coast and more precisely in Halltorps hage, a pasture dominated by old oak. One more find was made in 1889 in the north west part of Scania but has since then not been found in that province. Due to the realization that it was a rare species, the Greater Capricorn beetle received a preservation order in 1918 followed by the same protection given to 22 oaks in Halltorps hage in 1920 (Brinck, 1943). In the middle of the 20th century, potential findings of old galleries in oak made by *C. cerdo* were made in the adjacent provinces to Öland, namely Småland and Blekinge (Ehnström & Holmer, 2007). Old remnants from *C. cerdo* can be hard to distinguish from equivalent structures made by the Goat moth *Cossus cossus* larva though (Ehnström & Axelsson, 2002).

Ecology

In Sweden, the Greater Capricorn beetle is only found on oak, while it is also found on chestnut *Castanea sativa* in the Mediterranean (Ehnström & Axelsson, 2002). It is mainly found on trees that are rather isolated (Bílý & Mehl, 1989) and therefore exposed to sunlight which is another important factor (Brinck, 1943; Ehnström & Axelsson, 2002; Buse et al., 2007; Ehnström, 2007; Albert et al., 2012). The greater Capricorn beetle uses both the trunk and big branches to live in during the larval stage (Naturvårdsverket, 2009). Four characters that attract the Greater Capricorn beetle are, several hundred year old oaks, being the first

character, which generally have a greater trunk diameter (Buse et al., 2007; Redolfi De Zan, 2017), being the second character, with a large bark depth, being the third character and finally the occurrence of oak sap (Buse et al., 2007), a sugary liquid made by the tree. (Fig. 2)



Figure 2 Oak with oak sap. The oak sap is the darker area on the bark which will attract insects due to its' sugary content.

Greater bark depth creates deep slits in which females seems to prefer to lay their eggs (Döhring, 1955) while they are also reported to lay eggs in damaged parts of the tree (Ehnström & Axelsson, 2002). The eggs are hatched after approximately 14 days (Brinck, 1943) and the larva then lives inside the bark for a year before it makes its way into the sapwood and later into the heartwood (Brinck, 1943; Ehnström & Axelsson, 2002; Ehnström, 2007) as far in as 20-30 cm (Ehnström & Axelsson, 2002). This creates tunnels in the wood, galleries, which are 1.5-2 cm in diameter (Ehnström & Axelsson, 2002; Ehnström, 2007). When the larva is fully grown, in Sweden normally after three (Brinck, 1943) to four (Ehnström, 2007) years, it makes its way towards the bark surface only to back up in the wood again where it creates a pupal cell. The pupal cell is partitioned off from the galleries by a lid (Brinck, 1943), a calcareous operculum made by the larva. There it pupates, which takes place around August, followed by the hatching, approximately six weeks later, but it stays in the pupal cell for close to another year until late June when it enters the outside world (Brinck, 1943; Bílý & Mehl, 1989; Ehnström & Axelsson, 2002; Ehnström, 2007). During the day it usually hides in galleries in the trunk and appears first during sunset on warm summer evenings. This behaviour can be seen well into August (Bílý & Mehl, 1989; Ehnström & Axelsson, 2002; Ehnström, 2007) but the species is thought to be preyed upon by numerous bird species (Naturvårdsverket, 2009) so the season for individuals is likely rather short.

1.1.2. Oak

Oak history

Oak *Quercus* spp. is widespread in the southern parts of Sweden but has had a turbulent history. It established itself in Sweden slightly before and during the Atlantic period (c. 9000-6000 BP) along with other trees that are typical for the nemoral biome like elm *Ulmus glabra*, lime *Tilia cordata* and maple *Acer platanoides* (Hultengren et al., 1997; Niklasson & Nilsson, 2005; Lindblad & Froster, 2010). Forests with mainly old trees of oak and beech *Fagus sylvatica* were common in the middle ages (Nilsson, 1997). This was probably due to the intentional care of these species since they provided food in terms of acorns and beechnuts (Niklasson & Nilsson, 2005). In 1558, the Swedish king Gustav Vasa decided to take control over the wood resources in case it was needed in order to build ships for warfare (Eliasson & Nilsson, 2002). Those times came soon enough and since both Sweden and adjacent countries needed timber for ship construction the oak trees began to disappear.

During the time oak trees were considered property of the crown there was a strong discontent among the farmers who had oak on their land (Eliasson & Nilsson, 2002). The discontent resulted in farmers damaging trees in order to hurry on the succession. This created holes and dead wood, making the trees unfit for the navy and therefore less interesting for the crown. A common practice was also to pollard the trees which include cutting of branches and thereby decreasing the crown size. This was originally done to have fodder to give to the livestock during the winter but it was also a successful method if you wanted the crown smaller so that sunlight could reach the ground underneath. This was the case for many of the farmers who depended on the grass and herbs, that later became hay for the livestock, that grew on the land where the oaks stood. A combination of a growing population, farmers making their voices heard, a deficit of oak trees and changing politics made the government repeal the law. This in turn resulted in the old and mistreated oaks being cut down. In the years 1790-1825 oak trees, fit for naval standards, decreased by more than 80 percent. As a result of both the state giving up its rights and the farmers simmering discontent, approximately three million oak trees were cut during the years 1806-1835 (Eliasson & Nilsson, 2002).

Ecology

In Sweden there are both common oak *Quercus robur* and sessile oak *Quercus petraea* (Hultengren et al., 1997). Oak needs a relatively large amount of sunlight to rejuvenate, 30 percent of the daily sun exposure to be precise (Ranius & Jansson, 1999). The trees that are most prone to compete with oak due to their ability to shade the surroundings and their need of water are Ash *Fraxinus*

exelcior, aspen *Populus tremula*, birch *Betula* spp. and spruce *Picea abies* (Ranius & Jansson, 1999). This is reflected in the possible lifespan of oaks that will live on average 300 years in a closed forest, 400-600 years in pastures and as much as 1000 years in open landscapes (Niklasson & Nilsson, 2005). Old and robust oak trees with big branches indicate that there have been good growing conditions in one period or another when the oak grew up (Ranius et al., 2008). The Swedish meadows and pastures comprised optimal conditions for oaks to grow big and old and if the landowners hadn't already damaged the trees the old age alone made sure to create various habitats, like holes and dead wood, for all kinds of organisms to thrive in.

1.1.3. Inventory methods for the Greater Capricorn beetle

Methods used when monitoring the beetle include: VES (visual encounter survey), Collecting remains of predation along transects, CMR (Capture-Mark-Recapture), Surveying the exit holes, Artificial sap and Baited traps.

Redolfi De Zan et al. (2017) describes all methods mentioned above, reviewed CMR and surveillance of the exit holes and tested the remainders (VES, Collecting remains of predation along transects, baited traps and artificial sap) in Bosco della Fontana, Italy, in order to create a standard method for monitoring *C. cerdo*. Baited traps proved to be most efficient.

Even though Redolfi De Zan et al. (2017) describes how methods can be altered in order to fit certain population sizes, the population in Sweden is compiled to only a handful of trees which calls for other methods than just monitoring the current population. All methods described above require colonised trees with at least one generation of beetles emerged through exit holes. In order to register the larval presence in a recently colonised tree that lacks the visual traits we normally look for, we turn to the great olfactory senses in dogs.

1.1.4. Conservation detection dogs (CDD)

The foundation for conservation detection dogs were laid when kiwis *Apteryx* spp. were sniffed out by the dog Lassie on New Zealand in the 1890s (Hutching & Walrond, 2007) but it would take another hundred years before researchers started to use it for conservational purposes again (Lydersen & Gjertz, 1986). Several studies have been made during those hundred years on game birds in order to preserve their populations for hunting purposes (Gutzwiller, 1990; Dahlgren et al., 2012). Pests, in the shape of insects, have also been a target from the middle of the 20th century (Welch, 1990; Lewis et al., 1997; Pfiester et al., 2008; Hoyer-Tomiczek et al., 2016; Hoyer-Tomiczek & Hoch, 2020). There are several studies

made on the efficiency of dog search on chemical explosives (Gazit et al., 2005a; Gazit et al., 2005b; Harper et al., 2005) which has been done since the Second World War (Fyrton & Myers, 2001) and other similar concerns within the civilian use, regarding so called drug dogs or sniffer dogs (Jeziarski et al., 2014) for an example show their great importance in human society. From the beginning of this century, dogs detecting scat, mainly from mammals, has become increasingly used for estimating population size and distribution due to the great advances made by DNA research (Smith et al., 2005; Bennet et al., 2019).

“Conservation detection dog” is a collective term to distinguish dogs that are trained for conservation matters from dogs that are trained for a law enforcement context (Hurt and Smith, 2009).

The “Monitoring of insects with public participation” (LIFE11 NAT/IT/000252) was a Life project funded by the European Union and aimed at creating standard methods to be used throughout the EU in order to monitor five beetles in a scientifically consistent manner (Mosconi et al., 2017). The first official conservation detection dog to look for a beetle was a Golden retriever, trained to find larvae of the Hermit beetle *Osmoderma eremita* and by doing so, created the first study on dogs searching for an endangered beetle.

1.1.5. Dog qualifications

The working relationship between human and dog have diversified in the recent parts of the 20,000 years that we have been partners (Ruusila & Pesonen, 2004), making dogs’ the first domesticated animal (Galibert et al., 2011). The domestication of dogs has led to a selection, favouring dogs that are sensitive to human cues (Agnetta et al., 2000). The ability of the dog to react to arbitrary stimuli is of great relevance for dog training since the stimuli decided by us might not be of any intrinsic biological importance to the dog (Bensky et al., 2013).

Certain breeds are thought to be more suitable for this type of work than others (Welch, 1990). Jeziarski et al. (2014) concluded that German shepherds were one of the best breeds suited for sniffing out drugs whilst Terriers (Fox, Welsh, Jagd- and Jack Russel) did worst in terms of detection time, correct indications and false indications. In this study we used a breed called Irish softcoated wheaten terrier which is *not* chosen for this particular task but was instead the breed available and in care for, of the handler available. The owner and handler is a veterinary nurse at Nordens Ark and the dogs, Loka and Puma, have been trained in Nosework before the *C. cerdo* project started out in the autumn of 2019. Nosework is a sport, based on the work of sniffer dogs (Jeziarski et al., 2014), where the dog is taught to locate particular odours in outdoor and indoor environments in different settings (Lindhe & Nylund, 2017).

The breed of Irish softcoated wheaten terrier was used as a general farm dog and was assigned to hunt, guard and work the cattle (SCVTCA, 2016). Terriers

are in general bred for hunting (Galibert et al. 2011) and hunting dogs have been selected for their ability to track game and are therefore reliant on their olfactory sense (Quignon et al. 2012). Dogs in general learn to discriminate faster if the task is to smell rather than to look for an object (Welsh, 1990; Lewis et al., 1997; Pfister et al. 2008; Hall et al., 2013) but when it comes to olfactory tasks there is no conclusion yet on which breed is best suited for the job (Welch, 1990; Johnen et al., 2017) since both pugs (Hall et al. 2015) and cocker spaniels (Jeziarski et al., 2014) are better or just as good as the commonly used working dog, like retrievers and shepherds (Welch, 1990; Johnen et al., 2017). Fyrton & Myers, (2001) argued that there is more variation across breeds than is it between breeds when it comes to the olfactory abilities while Quignon et al., (2012) concluded that the olfactory capacities varies both individually and between breeds.

With this in mind our aim was to see if these particular dogs could successfully locate the scent of this particular beetle species larva in hopes that our method could be put to practice in similar situations for conservational matters.

1.2. Aim

The aim of this study was to evaluate a non-invasive method to which the goal was to see if two Irish softcoated wheaten terriers could locate oak trees with the Greater Capricorn beetle *Cerambyx cerdo* larva.

The following questions were asked: can the dogs learn to identify the scent of the Greater Capricorn beetle? And, can the dogs localize and signal for the larval scent on trees?

2. Method

2.1. Nordens ark

This study was mainly conducted at Nordens Ark which is a non profit foundation that works with conservation matters both in Sweden, where it is located, and internationally. The facilities are on the west coast of Sweden, in close proximity to the Skagerrak. They started working with the Greater Capricorn beetle *Cerambyx cerdo* in 2012 as a result of the conservation effort that was made by the Swedish government in 2009 when an action program was formulated for the species. The action program was initiated by the county administration board in Kalmar, on the east coast of Sweden. The aim then was to create a method to breed the beetle in an efficient way so that, later, adults could be put in appropriate environments, hoping the offspring would form new local populations. Since 2015, beetles, originally from Swedish localities, are being brought up at Nordens Ark (Naturvårdsverket, 2009). The method developed by Nordens Ark resulted in a breeding program, raising beetles in half the time it would take them under natural conditions. Adult beetles, brought up at Nordens Ark, being offspring from adults from Halltorps hage, have since then been placed in appropriately designed boxes, on suitable oak trees, in hope that they would lay eggs on the tree and by doing so, forming new local populations. This was done in the year of 2018, 2019 and 2020 in Kalmar county and Blekinge county. In order to tell whether the method is successful, exit holes would appear five years after the eggs were laid, as a result of emerging adults. A faster way of telling if this method is successful and whether there are live larvae in the tree or not, could be to use the olfactory sense of a dog.

2.2. Handler and dog experience

The handler started out training dogs in the year of 2000 and started competing with dogs in 2006 and has throughout used Irish softcoated wheaten terriers. Puma was born 2011 and is trained in Competitive Obedience, Rally obedience, Blood tracking, Agility, Nosework, Conformation show and tracking human scent. She was awarded with Best Swedish Allround Wheaten of the Year in 2014, 2017 and 2019 which means that she has fine awards for all three years in all the areas in which she is trained. Loka is the offspring of Puma. She was born 2015 and is trained in the same areas as Puma and has several awards including Best Swedish Blood Tracking Wheaten of the Year in 2017.

2.3. Experimental design

Training has been performed indoors in the handlers' home, outdoors within the Nordens Ark facilities, in natural populations on Öland and in areas on the Swedish mainland that might contain larva due to intentional release of adult beetles in Björnö. From October 2019 to July 2021, the handler did an average of 6 sessions a month. This results in a total of 124 training sessions held both indoor and outside. The experimenter was present during the outdoor training sessions.

During training where the experimenter was present, multiple parameters were recorded. In the Mosconi et al. study (2017), they collected data on the weather conditions; temperature, humidity and wind, in order to distinguish whether it could have an effect on the dog performance. We chose six additional factors: time spent in tree for the larvae, starting point relative to where the larva was hidden, larval size, type of container, height of the hidden location of the larva and targeting behaviour. The factors described were also thought to possibly affect the motivation of the dog. This was measured by collecting data on how many times the handler felt she needed to remind the dog on the task, how many times the dog lost its focus and how many times the dog indicated it wanted help by either whimpering or by searching eye-contact from the handler. Whimpering was not taken as a sign of motivational loss when in context of frustration when it was directed towards the target, being out of reach.

2.4. Equipment

Several different containers for larvae were used during search (Fig. 3).



Figure 3 1. Sifter jar with metal net on one side, 2. metallic tube cage with room for one larva, 3. plastic tube with room for one larva, 4. oak cages with room for cassettes (6) containing several plastic tubes and 5. metallic jars in three sizes with room for one larva.

2.4.1. Handling of larvae

Oak leaves and clean tweezers acted as mediums between hands and larvae. To exclude other odours surrounding the larvae, they were washed in temperate water during the period when the dogs learnt the odour of the larva. During the first trainings outdoor, the larvae were placed on the trunk of the oak for approximately one hour. This time increased gradually. The temperature had to be a minimum of 2°C in order for the larvae to be kept outside overnight to avoid jeopardizing the welfare of the larva. The larvae were enclosed with oak shavings in the jar in order to sustain the larval feeding needs when they were placed outside more than 24 hours.

Late November 2020 was the first time the larvae were kept on trees overnight followed by a training session observed by the experimenter. The time spent outdoors, as targets, before the training session increased from there on. In late march 2021 the time spent outdoors before a training session had reached two days.

2.4.2. Indoor training

Training indoors started in late October 2019 and has been performed by the handler only. The text concerning indoor training is a summary from the handlers' personal training notes. The handler did sessions on an average of seven times a month from October 2019 to July 2020. August and September 2020 was without any training due to the veterinary nurse workload.

Based on the handlers' perception of the dogs' response rate, the difficulty of task became increasingly difficult. This was done only to an extent where the dogs did not risk losing the motivation by being subjected to a too difficult task too early on in the process of learning.

The handler attended two courses held by Nordiska hund in order to get inspiration for further training methods for the project, one in the autumn of 2019 and one in the spring of 2020.

2.4.3. Learning the scent

The first task, starting in October 2019, was to distinguish the larval scent in small metallic jars (Fig. 3). Six jars were presented to the dog where one contained a larva while the rest were empty. The dog was then asked to choose the jar with the larva inside and was rewarded accordingly. This was repeated 10-15 times. When no mistakes were made on the first task, the handler introduced scents that would disturb the search in order to challenge the dog. Oak leaves, oak bark, NEKTON Drysophila breeding concentrate, oak shavings, both dry and moist separately, smoked ham, dry food and meatballs. This was done to assimilate the natural conditions when the larval scent might be in a context with other odours, and also to challenge the dogs with other, compelling scents.

In mid January (16/1) 2020 the handler started using other species larvae than *C. cerdo* as training on how to tell apart the correct scent from other larval scents. The species used were related to *C. cerdo* or likely found on oak and therefore important to tell apart from the target species. The species used were: *Rhagium sycophanta*, *Plagiontus detritus*, Musk beetle *Aromia moschatus*, *Pyrrhidium sanguineum* and *Phymatodes testaceus*.

2.4.4. Indication method

An indication is an alert made by the dog to indicate certain circumstances (Gilchrist et al. 2021). In our case, we wanted the dogs to alert when picking up the odour of *C. cerdo* larvae. The Mosconi et al. (2017) study used an indication where the dog sat towards the tree and barked when picking up the scent of *Osmoderma erimita*. The handler in our study chose a freezing on point indication, which means that the dog places the nose to the source of the scent as it can get and then "freezes up" by holding nose, head and body completely still.

The dogs were encouraged to re-check the target placement in order to get more treats.

Indicating on a coin was done to fortify the freeze behaviour while indicating. This was done only in a total of six sessions for this project since the dogs had previous experience of similar targeting behaviour through Nosework training. In late March 2020, but also during the two attended courses, the handler started using a search platform that is designed to teach dogs to search for specific scents (Fig. 4). The platform was then used to prolong the targeting freeze.



Figure 4 The platform designed to teach dogs to search for specific scents.

In order to prolong the targeting freeze behaviour further and to make the dogs more resilient to disturbances a treat was shown to the dog but was not given until the handler gave a verbal signal “Yes!” provided the dog kept freezing until the signal was given. This was not done in a context with the platform. This training method is called reversed enticement. It is a method where the dog is tempted by something desirable and has to withhold its’ instincts to grab the object until the handler gives a signal. This is meant to fixate the dog to the task and make it more attentive to the handler cues. The dogs were eventually subjected to more disturbances, by way of touching and movements while indicating, in order to make it more resilient and focused when exposed to similar or other disturbances in the field. The verbal signal was a part of all the steps in training

Late March 2020 was the first session outdoors when empty trees were introduced. This method was included until late May 2020 and it wasn’t until May 2021 that it became a regular method again. The pause was due to a lack of a set indication behaviour for when the dogs did not find the larval scent. The new

methods' aim was to reinforce the search drive and to stop the search when the majority of the tree was searched, according to the handlers' perception. The dog was called in and given a treat by the handler, a small distance from the tree.

In a context with trees containing populations of *Cerambyx cerdo*, the dogs were only allowed to search for short intervals when exposed to empty trees. The handler intended to reward the dogs for their search effort before any attempts at targeting occurred.

Smoke matches were used when the dog targeted from a different angle than directly towards the target on the trunk. The smoke visualised the wind direction and gave an indication to where the odour might end up. This in turn helped to evaluate the target placement as a correct or incorrect indication.

Training for a new indication method aimed at targets out of reach or diffuse odours was started out indoors in November 2020. The dog was subjected to an inaccessible toy and was rewarded when sitting and staring at it (Fig. 5).

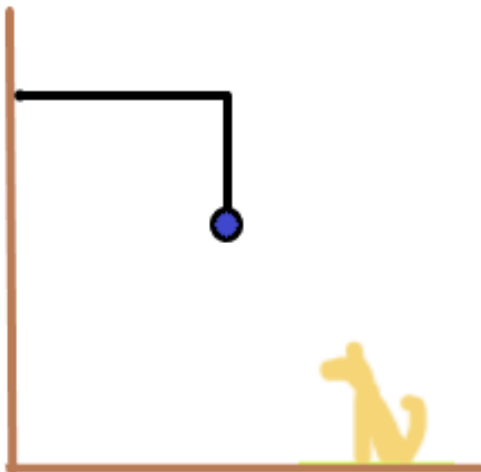


Figure 5 The final model depicting the targeting behaviour when the target was inaccessible. The dog is sitting down and staring at the ball.

In order to induce the sitting reflex in context with the inaccessible object the handler started off by rewarding the dogs even at an inclination of sitting down. A treat was later changed into a so called tulip ball (Fig. 6), a toy ball with Velcro closing that normally contains a treat inside. The reward was, later in the process,



Figure 6 A tulip ball that can be thrown as a ball when closed. All elements of this reward constitutes different reinforcements, from the vocal validation followed by presenting the ball to chasing, collecting and receiving the treat.

given in the form of another tulip ball thrown at the wall to which the dog was facing.

In April 2021, the dogs showed indication behaviour on visible objects on the tree trunk out of training context. Due to this behaviour, the targets were better hidden, starting in May 2021 in order to emphasize the odour targeting rather than the visual cue.

2.4.5. Motivation

Motivation is a key part in training dogs (Gazit et al., 2005b; Burman et al., 2011; Kis et al., 2012; Leonardi et al., 2012; Bensky et al., 2013) so in order to keep the dogs motivated the handler needs to make sure the dogs are rested, well prepared and that they find the task rewarding and fun. One factor was possibly hunger since training that was done before a meal and when the treat was highly desirable made the dogs appear motivated to complete the task. The treat was shown to the dogs before each training session to remind them of the award that awaited them. When there was a lack in motivation, the handler made sure to reward plentiful by playing catch with a tulip ball in order to increase the interest for the upcoming task. If the loss of motivation was consistent and several false indications were recorded continuously, the session was terminated.

The dogs showed no interest towards trees in the beginning of the outdoor training. This was solved by having the dogs search for treats on trees until the dogs started showing interest when searching on trees.

In an effort to record the motivation, data on certain behaviours were collected during the training sessions. Loss of focus was recorded e.g. when the dogs started searching elsewhere than on the tree, eating grass or getting caught up with passing people or other dogs. When the handler felt she needed to remind the dog on the task she gave a reminder by saying e.g. “cerdo” or “oops” in an encouraging manner. Reminders were recorded as well as the third sign of missing motivation which was asking for help. This was thought to be expressed when the dog looked at the handler and/or whimpered, which is described above in the Experimental design.

2.4.6. Outdoor training

Training outdoors was conducted without the experimenter until late April 2020 and was done only if the temperature was above 2 °C or below 20 °C. Harrison (2006) recorded the temperature during scat detection sessions being between 11-23° C. Rain and or extreme winds are not suitable weather conditions for training dogs according to Brooks et al. (2003) and Dahlgren (2012) which was also ruled out when planning for our training sessions.

Nine sessions indoors in between October and November 2019 made way for the first attempt at training outdoors. First with a metallic jar (Fig. 3) in a root cavity and on the second outdoor session, two larvae were put in each tea strainer. They were placed in a tree cavity and under a piece of moss low enough for the dog to reach in order for the dog to be able to perform the indication to the tea strainers by nose touch. The third outdoor training consisted of eight targets also placed in appropriate height for targeting by nose touch. The fourth outdoor training session resulted in a set starting routine where the dog was taught to sit and stay beside the handler until given signal “Okey, Cerdo!” and a hand motion towards the tree. On the fifth outdoor training in January the larva was left outside for more than 24 h in order to try and rid some of the human trace left behind when placing the larva on the trees. On the sixth and seventh sessions outdoors the handler brought a person to observe and act as a new element of disturbance. Late March 2020 the larva was placed in homemade metallic cages (Fig. 3) that were constructed to let more air, and thus scent, out in contrast to the metallic jars (Fig. 3) that only had an opening on one side. The placement of the samples was not possible to do in a random manner since we relied on the tree to have cavities in the bark or other suitable structures for the sample to stick to the tree trunk (Fig. 7).



Figure 7 A visible plastic tube with a larva in a bark crevice on an oak tree.

We exclusively trained on oak and the three main areas (Fig. 8) had ten or more suitable oak trees on which samples of larva could be placed. The dogs were mainly held on a leash due to the presence of cattle, unknown dogs, various vehicles, wildlife and people.



Figure 8 Areas in purple, orange and yellow represent the areas in which training was performed in the Nordens Ark manors.

2.4.7. Registered training sessions

Three main areas were used when training the dogs outside at Nordens Ark from April 2020 to July 2021 (Fig. 8). In late May 2020, the handler and experimenter visited the island of Öland where the dog searched on oak trees inhabited with *C. cerdo*. The scent was likely widespread around the trunk and there was no set point for the dogs to use the freeze behaviour on. The slert needed to be designed so that the behaviour was set for when the target was out of reach and the freeze on target did not work. The handler started thinking of a potential indication method in June 2020 (Fig. 9).

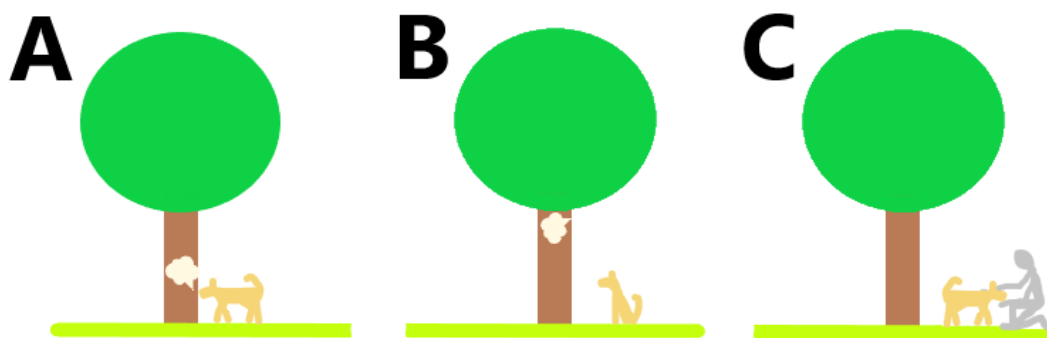


Figure 9 The final plan for different alerts depending on where the target was. A: Target reachable so the dog can alert by freezing on target. B: Target out of reach so the dog can alert by sitting and staring at it. C: No target is to be found so the handler decides when the dog has finished the search.

The training for targets out of reach started in late November 2020 after consultation from Ann Louise Ryrvik, a professional dog trainer. Late February 2021 was the first outdoor training with inaccessible targets. By then, it was also decided to avoid placing targets below 0,5 meter on the tree trunk since the search area naturally is higher on the trunk in relation to the infestation behaviour of the beetle.

Late March 2021 was the first outdoor session with several larvae used as one target. This was done to intensify the larval scent when the target was placed out of reach on the tree trunk. Oak cages were built by the experimenter, to hold cassettes with room for several larvae. Separate plastic tubes (Fig. 3) constructed to hold bed bugs *Cimex lectularius*, were placed in the cassettes. The placements of the larval targets, on trees, were grouped in to three categories. 1. Low, within reach; 2. High, out of reach and 3: Oak cage, out of reach by at least 20 centimetres.

In order to exclude the possible effect the handler would have on the dogs and thus the results, we performed a single-blind trial in April 2021. This was done with accessible targets only since the indication for inaccessible targets still required practice. The handler and the dog was unaware of the placement of the larva but the experimenter knew and was thus the one who had placed the larva on the trees 48 hours before the session. The larva were placed in plastic tubes with oak shavings and placed in crevices in the bark. The experimenter aimed at making them as invisible as possible for the handler while maintaining sufficient airflow for the scent to reach the dogs (Fig. 10).



Figure 10 An oak tree with a hidden plastic tube with a larva inside in an attempt to visually hide it from both the handler and the dog.

One dog at a time got to search 10 oak trees. Loka searched in one area and Puma searched in another. When the handler registered an indication she said “alert” and the experimenter replied “yes” or “no” based on the presence or absence of the target. In case the dog indicated on an empty site, the indication was ignored and the search continued until the dog found the correct location of the target although the results was collected as a false positive. If the dog made a correct targeting it was rewarded as usual with treats and play.

2.4.8. Methods that were discarded along the way

Six different *C. cerdo* larvae were used during the first period of training but only one larva was used each training session. The handler kept track of the individual larvae to begin with but this level of detail was soon discarded.

In January and February 2020, filter paper and parts of oak with larval scent was tried out as a possible substitute to live larvae but was discarded due to targeting on filter paper without larval scent, better indicating behaviour on live larvae and abundant larval access.

Early in the process of creating a set starting routine, the dog was told to sit, stay and wait for the handler to walk around the tree before the dog was allowed to search. This routine stopped once the experimenter started collecting data on the outdoor sessions since there was no longer a need for the handler to find the hidden target before letting the dog search for it due to its’ location being written down by the experimenter and because it was time consuming.

In the beginning of April 2020 the handler tested lifting the dogs to targets placed higher than their natural reach to see if the dog would show indicating behaviour while being held. This was discarded since the dogs refused to indicate while being held and because it would have been strongly biased and arbitrary as to how long the handler should insist on holding the dogs.

To avoid human odour the larvae was initially handled with nitrile gloves. This turned out to be a scent the dogs then associated with the larval scent so it was discarded in November 2020.

Similar to the Fischer-Tenhagen et al. (2017) study, a new indication for trees without targets was first thought to be visually straightforward by letting the dog stay at the target if thought to be present and by leaving the spot if the target was thought to be absent. This was rejected without a trial since experts (Michael Hedman), training dogs for conservation detection, found that it was too much responsibility for a dog to be given, having tried on dogs training for conservation purposes.

2.5. Data handling and analysis

The Mosconi et al. study (2017), with the aim to monitor the presence of Hermit beetle, was used as an inspiration when creating a template for data collection. This template was based on the true skill statistic (TSS), or the Hanssen-Kuipers discriminant, which is a method that aims to bring an alternative measure of accuracy to ecology than Cohen's kappa, being the standard statistical tool (Allouche et al., 2006). Results from TSS, was compared to the results of Cohen's kappa. TSS turned out to be a better choice for predictive models when distinguishing the distribution of low density species (Allouche et al., 2006). TSS is thus based on a presence or absence scenario and should be indifferent to the prevalence, i.e. the density of a population, or in our case, the frequency of correct indication behaviour on present objects. Bennet et al. (2019) noticed the use of this method in previous studies on dog detection since it also includes a presence or absence scenario. They chose to use three calculations from the TSS that are to be put in a confusion matrix (Table 1). A confusion matrix is the outcome of binary classification where there are two classes, positives and negatives. When put in a context of actual conditions, and the following outcomes, we can create a 2x2 table that gives us four outcomes. Put in our context when searching for larvae on trees, true positive means that there is a target and that the dog indicates. When false negative is logged, there is a target but the dog can't find it. On the contrary, true negative is when there isn't a target and the dog rightfully refrains from indicating and the last response, false positive, is when there isn't a target but the dog indicates as if there was one.

Both Mosconi et al. (2017) and Hoyer-Tomiczek et al. (2016; 2020) chose to use sensitivity, specificity and accuracy when evaluating performance for their dog search. These three measures are put in Table 2 which visualises the presence and absence scenario in a context with the resulting measure outcome.

Tabell 1 A basic confusion matrix that visualizes the idea of a binary classification.

	Positive condition	Negative condition
Test outcome positive	True positive	False positive
Test outcome negative	False negative	True negative

Tabell 2 Determination of sensitivity, specificity and accuracy by calculations from a confusion matrix. (Bennet et al. 2019)

Dog response	Targets	Presence	Absence
	Alert	TP	FP
	No alert	FN	TN
Sensitivity (true positive rate) True positives divided by the number of targets present		$\frac{TP}{TP + FN}$	
Specificity (true negative rate) True negatives divided by the number of targets absent			$\frac{TN}{TN + FP}$
Accuracy (correct indications divided by total number of samples)		$\frac{TP + TN}{TP + FP + TN + FN}$	
<i>Abbreviations: TP, true positive; FP, false positive; FN, false negative; TN, true negative</i>			

Bennet et al. (2019) evaluated studies with conservation dogs and came up with a framework for evaluating this kind of work. The framework consists of five different aspects: sensitivity, precision, effort, cost and comparison with alternative methods. Sensitivity is the true positive rate while precision is the measure of all indications made on true targets or the ability to distinguish the right scent from the wrong scent (Bennet et al., 2019). We chose to include specificity and exclude precision due to the design of our method. The training that would strengthen precision was exclusively done indoors during the data collecting period. Specificity is the measure of all correct negatives and used both by Mosconi et al. (2017) and Hoyer-Tomiczek (2016; 2020). Effort is described as time spent on searching divided by the area searched. Mosconi et al. (2017) described effort as time divided by number of trees searched on. Since the dogs were on a leash and presented to one tree at the time we will also present effort as time divided by all trees searched. On the one hand including the time it took to go from one tree to another and on the other hand presenting the average time it took to search on tree. Cost aspects will be generally discussed in the light of our study and other similar studies. A comparison with alternative methods will be done in the discussion.

All of the observations will be presented in four periods in order to sort the data into comparable units. Since the training period include recurring pauses, dividing the period enabled the training periods to be compared without the pause. The division aims to compare the four periods in terms of development and the dogs are separated in order to compare their individual progress. The first period

was the 5th of May to the 6th June 2020, the second was the 30th of October to the 30th of December 2020, the third was the 25th of February to the 23rd of April 2021 and the fourth period was the 7th of May to the 24th of June 2021.

Influencing factors and motivation is presented in relation to the time it took for the dogs to find the target.

The specificity is based on the frequency of true negatives and false negatives and is lacking collectively in period two and three. Both periods two and three excluded empty trees and all targets were reachable. Empty trees were introduced in the first period and reintroduced in period four which were required for a true negative outcome.

The comparison of sensitivity, specificity and accuracy between periods was run with the Kruskal-Wallis test to look for differences and the Man Whitney U-test was used to see which periods that differed. The Man Whitney U-test was also run to compare the two individual dogs' performances of sensitivity, specificity and accuracy. To test if temperature, humidity, wind, time spent in tree, starting point, type of container, larval size and the height of the target on the tree trunk affected the search time, an ANOVA (Analysis of variance) was run. Lastly, a regression analysis was made on the motivational measurements, namely the number of reminders by the handler, the number of time the dog lost focus and the number of times the dog asked the handler for help, to see if they correlated with the search time. All statistical calculations were made in the statistical software Minitab.

3. Result

3.1. Differences between periods

The first, second and third period all had maximum results concerning sensitivity for both dogs (Table 3; Table 4). The specificity between period one and four for Loka was not significantly different (Table 3).

Both dogs showed a higher accuracy in period one compared to period four (Loka: $P=0,04$, $W=2626$, Table 3; Puma: $P=0,002$, $W=2317$, Table 4). Those are the only periods that could be compared since statistics cannot be done on groups without variance.

Tabell 3 Lokas' results measured in Sensitivity, Specificity and Accuracy based on the frequency of TP; true positives, TN; true negatives, FP; false positives and FN; false negatives.

Period	Trees	Targets	TP	TN	FP	FN	Sensitivity	Specificity	Accuracy
1	43	40	40	-	3	-	100	96	93
2	55	55	55	-	-	-	100	-	100
3	51	51	51	-	-	-	100	-	100
4	63	53	42	6	13	2	79	75	76

Tabell 4 Pumas' results measured in Sensitivity, Specificity and Accuracy based on the frequency of TP; true positives, TN; true negatives, FP; false positives and FN; false negatives

Period	Trees	Targets	TP	TN	FP	FN	Sensitivity	Specificity	Accuracy
1	43	40	40	2	1	-	100	100	97
2	55	55	55	-	-	-	100	-	100
3	51	51	51	-	-	-	100	-	100
4	53	45	38	3	9	2	84	60	77

3.2. Difference between dogs

Out of all the tests, only the sensitivity was significantly different between the two dogs' performances. Puma was showing higher sensitivity than Loka ($P = 0,02 \times 10^{-6}$ $W = 2833$) in the fourth period.

The blind test on targets within reach resulted in 9 out of 10 found larval targets for Loka and 9 out of 9 targets for Puma.

3.3. Influencing factors

The protocol had room for multiple factors that could possibly affect the result. Time spent in tree before training, starting point relative to the target, height of the target, type of container in which the larva was held, size of the larva, temperature, humidity and wind were all tested in relation to the time it took for the dog to find the target.

One significant correlation was found in Lokas results (Table 5). It took less time to find low targets (mean=48 sec, SE=3,7, P=0,002) compared to targets in oak cages (mean=125 sec, SE=38, P=0,0001).

Two significant correlations were found in Pumas' results. At a humidity of 54 % it took significantly longer to find the target (P=0,001) compared to all other humidity measures. Also, at the same session, when the target had spent 1190 min in the tree it took significantly longer to find the target (P=0,001).

Tabell 5 Result of ANOVA of Lokas' time versus temperature (C°), Starting point, type of container and larval size. Df (Degrees of freedom) is the number of subgroups (eg. Small larval size) minus one. P-value set at a confidence interval of 95 %.

Factors	Df	P-Value
Temperature (C°)	12	0,245
Humidity	17	0,359
Wind	6	0,693
Time spent on tree	17	0,407
Starting point	3	0,980
Type of container	3	0,153
Larval size	4	0,373
Height on trunk	3	>0,001

3.4. Motivation

In an effort to measure the motivation we observed three behaviours that might have had a connection with the time it took to find a target. The three measurements were; number of reminders by the handler, number of time the dog lost focus and the number of times the dogs asked the handler for help by looking at the handler. Reminders showed a positive dependence with time, meaning that we can expect more reminders as time increases for both dogs (Fig. 11).

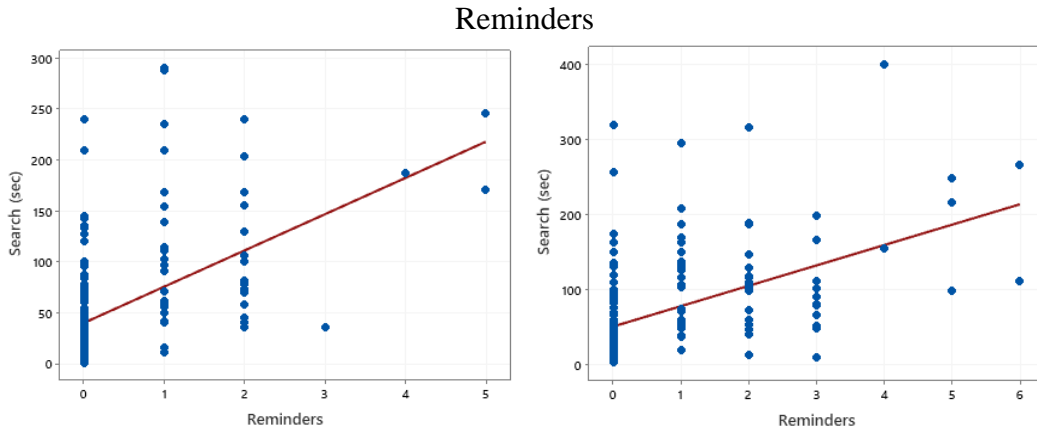


Figure 11 Result of Lokas' (to the left) and Pumas' (to the right) time in search versus the number of reminders that the dogs were given by the handler. The R-value was 0,53 for Loka and 0,49 for Puma. Both results had a P-value of less than 0,001.

The correlation between loss of focus and time spent in search was not as strong as the correlation between the number of reminders (Fig. 12). The correlation was thus significant but weak.

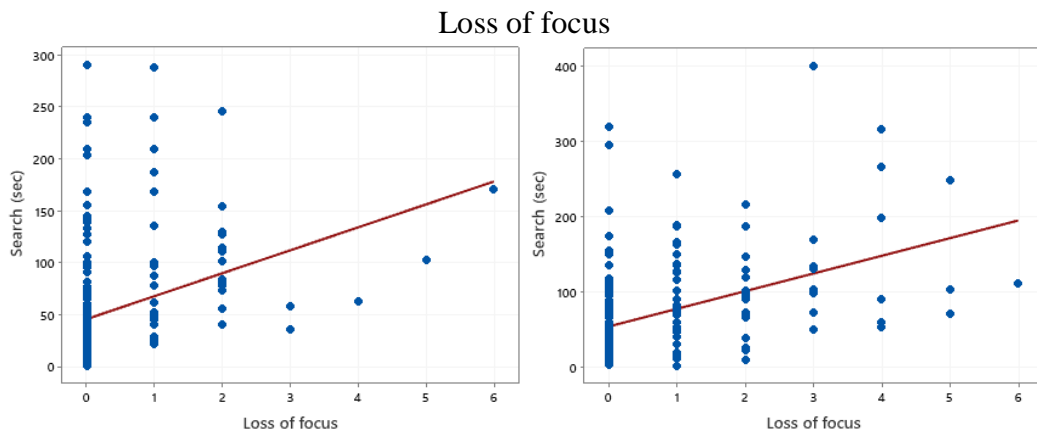


Figure 12 Result of Lokas' (to the left) and Pumas' (to the right) time in search versus the number of times the dogs lost focus on the task. The R-value was 0,33 for Loka and 0,43 for Puma. Both results had a P-value of less than 0,001.

Out of all three motivational factors, the search for help showed the strongest correlation with time spent in search (Fig. 13). This means that we can expect the dogs to search more for help as time goes by.

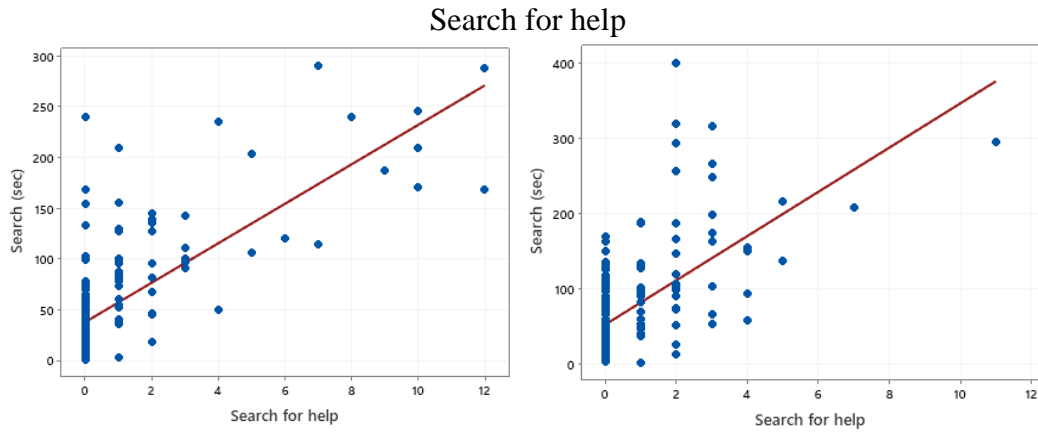


Figure 13 Result of Loka's (to the left) and Puma's (to the right) time in search versus the number of times the dogs asked for help. The R-value was 0,74 for Loka and 0,59 for Puma. Both results had a P-value of less than 0,001.

3.5. Efficiency

The average time to search one single tree was 53 seconds for Loka and 1 minute and 10 seconds for Puma. The whole training session divided by the number of trees resulted in 3 minutes and 10 seconds for Loka while it took Puma 3 minutes and 33 seconds.

4. Discussion

When comparing the four periods regarding sensitivity, specificity and accuracy, both dogs had a significantly higher accuracy in the first period.

Comparing the two dogs gave a higher value of sensitivity for Puma. The lack of variance prevented some of the groups from being compared with one another. Specificity required training on empty trees which was lacking in both period two and three.

Potentially influencing factors were different for the two dogs. Loka had significantly shorter searches for reachable targets compared to, out of reach, oak cages. Puma on the other hand needed more time in search when the humidity was at 54 % and when the target had spent 1190 minutes (19 hours and 50 minutes) in the tree before the search.

The motivational factors that showed the strongest correlation with time in search was the number of times the dogs asked for help and the number of times that the handler reminded the dog on the task. Loss of focus had weak correlation with time in search.

To evaluate the method and the result we have chosen five measures; Sensitivity, Specificity, Accuracy, Effort, Cost and Comparison with other techniques (Bennet et al., 2019). These will be discussed in the light of our result and dog behaviour.

4.1. Sensitivity (TP/TP+FN)

Sensitivity is the measure of all true positives divided by all targets available.

Puma showed a higher sensitivity rate than Loka in the fourth period. According to Bennet et al. (2019), the importance of sensitivity depends on the requisites. If the presence of the target scent is sufficient, without the knowledge of all potential targets in that spot, sensitivity is less important. This is the case for our study since the first goal is establishment of the beetle without the need for exact numbers. All other periods regarding sensitivity was not comparable due to the lack of variance. This was because sensitivity was logged one hundred percent in all three periods for both dogs. This in turn was because the target was searched for in such a manner that the dogs would find it eventually without risking the loss

of motivation should they not have found it. Lewis et al. (1997) found that training with a lower number of targets, relative to the number of empty sites, gave a significantly lower rate of true positives. Thus, having a high number of targets aimed at creating an encouraging environment for the dogs. Given that the dogs showed a high number of true positives on their blind test, this might have been a result of that.

4.2. Specificity (TN/TN+FP)

Specificity is the measure of all true negatives divided by all empty trees. Both dogs had results for period one and four. Period two and three lacked empty trees and had thus no results. Puma had one hundred percent specificity in period one and could thus not be compared to Lokas result ending up on ninety-six percent. The supposedly different result between the dogs in period four, and between period one and four for Loka, is not significant due to the low number of empty trees.

One of the reasons for the relatively low numbers of specificity in the fourth period is the high numbers of false positives. Both the freeze indication and the “sit and stare” were more or less associated with visual targets. Even though the dogs indicated on hidden larvae, they were also keen on indicating visual objects that was found on the tree trunk regardless of their scent. False positives were also associated with lack of motivation and thus an effort to be rewarded rather than to actually search for the target. Dogs might also be more prone to indicate if the targets are scarce due to the urge for a reward (Harrison, 2006). This might also have been a reason for the high number of false positives since the measure of specificity depended on training on trees without targets.

The majority of the targets were visible on the tree trunks due to results from previous studies saying that dogs use their sense of smell rather than their sight to locate objects (Welsh, 1990; Lewis et al., 1997; Pfister et al. 2008; Hall et al., 2013). This was true for sessions where the dogs started searching seemingly head on a reachable target but still decided to search around the trunk before indicating. On the other hand, the dogs showed a strong visual association when they were expected to “sit and stare” on a target out of reach. Small metal plates with oak ID numbers were out of our training context but the dogs clearly indicated them, regardless of their lack of larval odour. When the concept was introduced to the dogs, the focus was on visual stimuli rather than olfactory cues (Fig. 5). This might be a reason for why the dogs indicated on visually distinctive objects on the trunk without searching for the scent first.

Once a behaviour is learned it is difficult to teach the dog that the correct way is to do the opposite of the previously correct behaviour (Benksy et al., 2013). This in turn can be applicable when carryover effects are discussed because of the

strong associative learning that gets stronger with each replicate (Benksy et al., 2013). In April 2021, it became increasingly difficult for the handler to tell whether the dogs sat and stared at the tree as a true positive or if it was in fact a false positive. This in turn made it difficult to reward the dogs in a distinct manner depending on the presence of a target or not. More targets within reach became the new objective in order to encourage a detailed search on the tree trunk. It was thus decided to temporarily stop training the dogs in June 2021 when the dogs chose to sit and stare before showing any tendency of having caught the scent. This gave the handler an opportunity to come up with a plan to help the dogs get back on track.

4.3. Accuracy ($TP+TN/TP+TN+FP+FN$)

Accuracy is the measure of all correct indicating behaviours divided by all available targeting behaviours. Accuracy differed between period one and four for both dogs. The high number of accuracy in the first period was likely due to the training setup mentioned in the sensitivity part. The dogs were challenged in a manner where they did not risk losing the motivation because the task would have been too difficult. The apparent loss of accuracy was thus due mainly to the new indicating behaviour called “sit and stare” since it resulted in a high number of false positives which affected not only the specificity but also the accuracy.

4.4. Effort

We chose to present the effort both as the average time it took to search one tree and the equivalent time including the time it took to go from one tree to another. The latter value is interesting in the light of the time required for a search session.

The average time to search a tree alone was 53 seconds for Loka and 1 minute and 10 seconds for Puma. This can be compared to similar searches for *Anoplophora glabripennis* that took on average 2 minutes and 42 seconds (Hoyer-Tomiczek, 2017) and searches for *Osmoderma erimita* that on average took 6 minutes and 50 seconds (Mosconi et al. 2017). Including the distance between the trees, and the occasional water break, it took Loka 3 minutes and 10 seconds while it took Puma 3 minutes and 33 seconds. This result was also affected by the time spent on rewarding the dogs, which varied from time to time.

Factors that effected the search time were the height on the trees for Loka and humidity and target time spent in tree for Puma. It took significantly longer for Puma to find a target when the humidity was 54 % and also when the target had spent 1190 minutes in the tree before search. The two influencing factors occurred on the same training session and the time in search was most likely higher due to

other factors than the two mentioned factors alone. No other trend was found regarding humidity or the time spent in the tree before search. The indicating behaviour where the dogs are supposed to sit down and stare at the target had been introduced shortly before this session. Even though the dogs seemed eager to indicate on the larva the search time was not stopped until the dog showed the sit-and-stare behaviour.

Motivation was also something that effected the time in search. Loss of focus had a weak correlation with time in search compared to number of reminders and the number of times the dog asked for help. The loss of focus might be due to the arbitrary nature of distractions. The sudden scent of a wild animal or a passing dog might create a short distraction and loss of focus but might not necessarily come from fatigue.

Training intensity has varied in the studies examined for this project and daily training has been a minimum for five studies (Williams & Johnston, 2002; Brooks et al., 2003; Gazit et al., 2005a; Gazit et al., 2005b; Pfiester et al., 2008). Training four to five times a week was done by three studies (Smith et al., 2005; Mosconi et al., 2017; Welsh, 1990) and training one or two times a week was least common (Fisher-Tenhagen et al., 2017; Gagnon & Duré, 1992) of those who reported training intensity. The average training intensity for this study was six sessions a month which would correspond with the latter group. In reality, the training sessions were performed daily in certain periods and weakly in other periods. The pauses in training all together also affected the average number of training sessions. Since the handler did not train the dogs full time, this setup was the outcome of available time for practice in between working hours at Nordens Ark. Williams & Johnston (2002) found that dogs will remember an odour they have targeted and need on average two sessions to regain the performance they originally had if subjected to a period without any practice. They also found that dogs will learn new odours faster if they already have a repertoire of odours they can target. Both dogs were well experienced with Nosework and had thus prerequisites to handle odours.

Many hours went into keeping the larva fit when not used, but also the preparation for when the larva was put into tubes, stuck on tree crevices and taken down again, took time. This could have been avoided by using filter paper instead of live larva. There are several advantages of filter papers regarding the efficiency and work load. The larva cannot be exposed to extreme temperatures, it needs food and must be enclosed in a manner that prevents it from escaping but ensures air to go through in order for their scent to spread. Although Mosconi et al. (2017) used filter paper with the target odour, both Brooks et al. (2003) and Lewis et al. (1997) indicate the importance of a scenario as close to natural conditions as possible regarding the odour intensity. Filter paper was ruled out in our study since the dogs targeted the filter papers own odour. Brooks et al. (2003) discussed

the dogs' odour detection threshold as being dependent on how training had been conducted. Our aim was thus to mimic the natural intensity of the odour by using live larva. By decreasing the scent gradually when hiding the larva on different heights and on different bark depths the detection threshold would eventually correspond to natural conditions.

4.5. Cost

Bennet et al. (2019) highlights the importance of putting the project in an economical perspective since scientific projects often are restricted by a budget. The importance of cost effectiveness will vary with the availability of invested people who are willing to work for free, or if the project is dependent on charging experts.

Dahlgren et al (2012) is discussing the obstacles regarding the cost of a trainer and the possible investment you would have to put in as a student and or experimenter. The project time spans a considerably shorter time period than the life of a dog which means that the purchase of a dog might not be the evident choice while hiring an already trained dog is expensive. Our experimental design allowed for spared expenses while using moderately trained dogs. This setup was particular to our circumstances and the economical benefits are therefore not generally applicable. The handler in our project expressed, in hindsight that the benefits with using an even more experienced trainer might have been worth the cost due to the vast set of challenges we faced. Professional dog handler teams are in several cases (Browne et al., 2006; Harrison, 2006; Duggan et al., 2011; Paula et al., 2011) more expensive than human equivalents but the efficiency of dog search can prove more economically effective if used in combination with other inventory methods.

Time spent on looking for a target of some kind has been considerably shortened with the aid of dogs (Browne et al. 2006; Paula et al., 2011). Harrison (2006) found that detection dogs gave 10 times the number of finds than the other available methods when looking for bob cats. Although expensive, hiring a dog detection team seems to outweigh the cost due to its effectiveness (Paula et al., 2011).

4.6. Comparison with other studies

Three other studies were found that trained dogs to search on tree trunks for beetles (Mosconi et al. 2017; Hoyer-Tomiczek et al. 2017; Hoyer-Tomiczek et al 2020). Many other studies were found on sniffer dogs and training dogs for odour detection.

When searching for explosives, if the dogs were exposed to an area without targets, their motivation not only gradually sank during the trial, but when targets were placed in the same area later on, the success rate was lower than that of another area, in which there had been continuously scattered targets (Gazit et al. 2005a). This is especially relevant for dealing with difficult target odours. Gazit et al. (2005b) showed the importance of high densities in numbers of targets in order to have good performance results. This behaviour has been discussed since the 1930's. The conclusion is that the context can alter the search behaviour significantly in various species of animals (Von Uexkull, 1934; in Shettleworth, 1998; Tinbergen, 1960; in Gazit et al. 2005a; Bouton & Ricker, 1994). This highlights the dependency on motivation if the search is going to be trustworthy. The method thus has to take into account the possibility of tainting an area, at least for a couple of days time, with the memory of it being something not worth while searching, according to the dogs previous experience.

One of our motivational measures was reminders, which were given by the handler in a manner that we can call them encouragements. Too much praise throughout the trial can have a reverse effect, disturbing the search process and thus misleading the dog (Henschel et al., 2020). Both reminders by the handler and the search for help by the dog went up as time increased. The search for help had a slightly higher R-value than reminders which indicate that the handler did not use too much praise, or encouragement since the dog asked for help more than it received.

Fischer-Tenhagen et al. (2017) used a 50/50 absence presence target design, when working with herb odours, while Hoyer-Tomiczek et al. (2016) used 25/75 absence presence. Both methods are scientifically preferable since they aim to create, firstly, a non-biased search outcome and secondly, natural conditions regarding rare species. According to the work of Gazit et al. (2005a; 2005b) we chose to use a skewed absence presence design to maintain the motivation of the dogs i.e. having more presence targets than absent ones.

Studies have generally seen a decrease in performance the longer the working day and the harder the searching task (Bensky et al., 2013). To make sure the motivation is high; some studies had the dogs tested before deciding if it was fit for the task by seeing how keen it would be to freely approach a toy or food (Kis et al., 2012). Another way of creating more favourable conditions was to have an experienced dog. Dogs that had undergone some sort of training were better at solving problems given to them than dogs with no training (Marshall-Pescini et al., 2008). The handlers' consideration to use professional dogs is discussed in the cost section above.

Food or treats were commonly used throughout the studies examined for this project (Williams & Johnston, 2002; Brooks et al., 2003; Gazit et al., 2005a; Pfiester et al., 2008; Kaminski et al., 2012; Hoyer-Tomiczek et al., 2016; Fisher-

Tenhagen et al., 2017; Mosconi et al., 2017; Hoyer-Tomiczek et al., 2020) and is also the most common reward for bomb dogs (Fyrton & Myers, 2001). Positive reinforcement is standard when training dogs (Fisher-Tenhagen et al., 2017). Walker et al. (2006) confirmed that it is indeed more effective to use treats and praise when the desired behaviour is shown rather than to use electro shocks and water deprivation to call out the unwanted behaviour.

Welch (1990) on the other hand argued strongly for the use of praise only, also employed by Gagnon & Duré (1992), since the risk of running out of food or lack of hunger in the dog would leave the dog without motivation to continue working. Thus, they excluded food from the training all together to make sure the dog would work for praise alone. Williams & Johnston (2002) solved the latter problem by keeping the dogs at 85-95% of their free feeding bodyweight, ensuring the motivation to be high for food rewards. The dogs in the Gazit et al. (2005a) study received 60 % of their food after training sessions were held and the rest during practice. The handler in this study fed the dogs different amounts of food depending on how soon the training session would follow. If a session was to be held in the morning she would reduce the first feeding to 50-75 percent. This was not done to increase the motivation, as was mentioned above, but rather to make sure the dogs stomachs weren't too full, making it uncomfortable for them to work with their bodies.

4.7. Ethics

Three types of judgements is presented in Batesons' (2005) work that evaluate the ethical perspective and thus the scientific and societal gain put in contrast to possible animal suffering. The number of dogs is too small to draw any scientific conclusions on dog training but if the dogs successfully locate larva of the Greater Capricorn beetle, we will get a better understanding on how to reintroduce the Greater Capricorn beetle to suitable areas. Regardless of the outcome, the result will give us an indication on how wise it is to keep using these dogs in order to locate the Greater Capricorn beetle. Human society will benefit from an increased biological diversity if the method can be put to use when re-establishing the beetle on the Swedish mainland.

Nosework as a sport has proven to be beneficial for dogs' mental health (Duranton & Horowitz, 2019) which can advocate for our project, having had an overall positive effect on the dogs. Reverse enticement though, can be a questionable method when training dogs. The method takes advantage of the frustration created in the dog when a treat of some sort is presented only to be swiftly removed when the dog tries to take it. The aim is for the dog to understand that the treat is really only available when the handler gives a signal. The handler

in this project had used it before on the dogs in other contexts. The method was thus not introduced for this training in particular.

When sniffing, dogs can accidentally receive allocating material in their olfactory system which can be harmful (The handler, verbally).

Apart from ethics concerning the dogs, the welfare of the larva was considered throughout the project. No larva was killed during the training or in between training sessions. Based on the current knowledge on insect perception, experiments with insects do not require ethical permission. Regardless of this, it felt intuitive to try and handle the larva with care to minimize possible discomfort.

4.8. Obstacles

4.8.1. Communication

As a result of human-dog relationships during thousands of years, dogs learned how to communicate in order for humans to understand (Miklósi et al. 2003). This is particularly evident when the dog desires an object which is communicated through alternative gazes (Miklósi et al. 2000) which is not found in comparative studies with wolves since they try to solve the obstacle themselves (Miklósi et al. 2003). The dogs had been trained through previous tasks, in other contexts, to create eye contact with the handler when showing/targeting. Thus, when the dogs was first faced with the task of searching naturally inhabited trees on Öland, eye contact with the handler became increasingly common throughout the sessions when the dog was unable to reach the target and no other indication behaviour was introduced. Whether it was because of previous training and thus induced behaviour or due to the natural instinct to search for help by looking at the handler (Kaminski et al. 2012) is hard to tell. In spring 2021 when the new targeting method was trained for it was evident that the first instinct was to look at the handler instead of staring at the ball which was the task in order to get a treat (Fig. 5). It was also evident that the dogs associated the handlers basement with sitting practice, being the location where sit and stare training was introduced. Ashton & De Lillo (2011) mentions this, as associative processes play a bigger role than the knowledge about object location.

4.8.2. Choice of dog breed

Dahlgren et al. (2012) states the importance on choice of breed for conservation work but mentions that individuals within a breed have further traits that can impact the training success.

Storengen & Lingaas (2015) found that Irish softcoated wheaten terriers had higher noise sensitivity than then 17 other species tested. Age was also a factor

that increased fear of high noises (Storengen & Lingaas, 2015) which might be worthwhile considering when deciding upon a dog individual.

Picking up on different human cues varies between dog breeds and dogs bred for cooperative work are better at following and interpreting human signals (Wobber et al., 2009). The Irish softcoated wheaten terrier is bred for independent work rather than cooperative work (SCVTCA, 2016). This might have worked in our advantage since the dogs would be less prone to interpret the participants' unintentional cues.

4.8.3. Odour properties

Throughout the project, the handler was faced with challenges such as working out the larval scent properties since it is an important factor to understand (Gutzwiller, 1990). Earlier in the *C.cerdo* project another SLU student (Svensson, 2016) tried to distinguish pheromones from adult Greater Capricorn beetles. No pheromones were found but the behaviour indicated that the males are drawn to the females by some factor. No studies were found on odour properties of the greater Capricorn beetle larva. Observations from our training with live larva on tree trunks suggest that the scent travels downwards.

If the wind is strong and the larva is high up on the tree trunk, the scent might travel in the air and land away from the tree or even on an adjacent tree (Fig. 14).

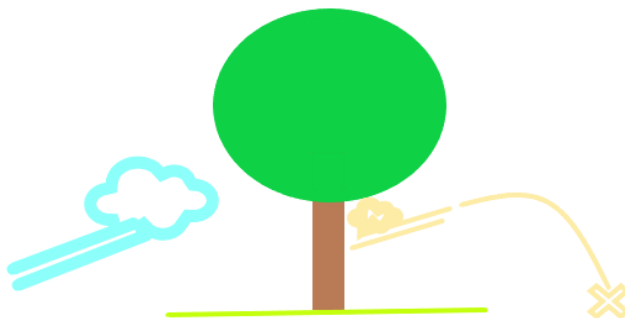


Figure 14 A sketch on how the scent might travel from the tree to a nearby spot due to wind conditions.

This might result in a false positive on an empty tree (Hoyer-Tomiczek et al. (2016) In order to pick up the scent, if landed elsewhere, a search method called “cinnamon bun search pattern” was suggested to the handler from experts (Michael Hedman). The dog starts to search in a circle around the tree and moves in towards the tree trunk in a spiral manner (Fig. 15).

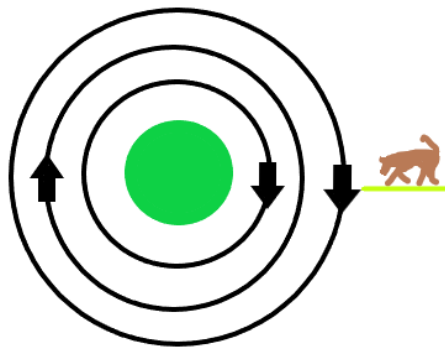


Figure 15 The so called cinnamon bun training which was a suggested method in order to pick up the odour that might have ended up beside the tree. The circles represent the movement patterns the dog would follow in order to catch the scent when circling towards the tree.

Gutswiller (1990) warns of too strong winds for dogs search on game birds since the scent might greatly disperse. But wind might also have beneficial effects on the search. Hoyer-Tomiczek et al. (2020) favours the value the wind might have on the odour distribution seeing as their results got better when the wind was moderate (2 m/s) rather than weak (0,5-1 m/s). On the other hand, Pfiester et al. (2008) argued that the lack of airflow in their experimental setup, indoors, was one of the reasons for their success.

4.8.4. Training approach

In hindsight, the handler had many ideas on things that could have been done differently. Training with a so called Kong was one and is standard in international contexts for sniffer dogs. The Kong is a conical, firm rubber tube toy. It comes in different sizes and has a hole in the middle which enables treats to be hidden inside the Kong (Kong Company, Golden, CO, Branson and Rogers, 2006). The Kong comes in different sizes and scents but for this purpose it has to be the Kong Classic red. It has its own particular scent and can be hidden in suitable places for the dog to sniff out. The Kong can later be cut into smaller pieces to increase the difficulty when hidden. In order to associate the task with other olfactory uses the Kong can later be replaced with a preferable target odour. The handler was familiar with the use of Kong. Since it was not used in the Mosconi (2017) study however, which was the outset of the study; the use of Kong was limited to two occasions and ruled out by the handler since it would have been greatly time consuming.

The intention in this study was to include both blind tests and double blind tests. No double blind was performed though due to the difficulties we faced with the sit-and-stare indication. A human gaze, a nod or even just eye movements towards the search area can be enough aid for the dog to find a hidden object (Miklósi et al., 1998; Agnetta et al., 2000 Ittyerah & Gaunet, 2009; Duranton & Range, 2017). Mosconi et al. (2017) used a, for the dogs, unfamiliar person to perform the double blind tests. Buttelman & Thomasello (2012) found that human emotional cues can guide the dog to the correct target which also makes for a

good reason to include a person the dogs aren't familiarized with. Kaminski et al. (2012) also found that even though eye contact will strengthen the intent in a number of communicative signals between dogs and humans, dogs will understand intentionality even without eye contact. Lit et al. (2011) tried out a scenario where the handler of the dog was given false information on where the targets were hidden. This resulted in false positive reactions from the dog on the occasions where the handler thought there were targets present even though there weren't any and even though the handler intended to be neutral.

Synchronization is common among many animals but is not assumed to occur between different species except between humans and dogs. Dogs' can adjust their behaviour to other dogs which in turn enables them to learn by observing others (Duranton & Gaunet, 2015). Studies suggest that dogs, opposite to wolves, have developed a way to interpret human cues in goal-directed contexts (Agnetta et al., 2000).

Hall et al. (2013) chose to do numerous control trials in order to rule out any unwanted interference from the surroundings. This might have affected the learning rate but the experimenters wanted to make sure no human cues guided the outcome as reported in other studies (Miklósi et al., 1998; Ittyreah & Gaunet, 2009; Lit et al., 2011; Buttelman & Tomasello, 2012). In this study there were only one control trial, which might have kept that sort of negative interference to a minimum.

Many things can affect the learning efficiency in the dog, the chosen acoustic stimuli being one (Bensky et al., 2013). The handler developed the acoustic stimuli as well as the body language, including hand movements, through out the training period in order to create an exciting and clear start for the dogs.

By using the word "Cerdo" when initiating a search, the assumption was that the dog can understand the meaning of the word in order to look for the desired target. Kaminski et al. (2004) suggest that this is indeed the case based on trials when dogs were asked to pick up both known and unknown objects and therefore also differentiated between them. Similarly, dogs have been shown to understand the goal of their work (Benksy et al., 2013).

Martin & Bateson (2007) is asking the question "What is ideal and what is practicable?" .When designing this project, it was important to maximise the dogs opportunities to learn. This mindset was due to the handlers' own experience in dog training. Hall et al. (2013) had trials with blind testing regularly through the training period and noticed a possibly slower learning rate than if focus stayed on reinforcing the correct behaviour. Our focus was the opposite, meaning we took as many opportunities as possible to give the dog positive feedback rather than risking the confusion created when exposed to a choice without a clear response.

The sessions were held when practicably possible and were therefore spread out on weeks and months during the one year and nine months the project was

supervised. This might not have been optimal since several studies show that more frequent training will increase the proficiency (Welsh, 1990; Brooks et al., 2013) while these studies like us made sure to keep each training session short to avoid fatigue. The handler took courses, from various dog experts, when faced with difficult challenges through the entire project period. In order to standardize the method in conservation dog studies, Bennet et al. (2019) created a list of factors that should be included in the project layout. All these factors were included in our project as the aim should always be to create a replicable study.

4.9. Conclusion

Although dogs understand human cues to a certain extent, they do not grasp our research intent which makes it important to try to remove possible bias. This is not easily done since dogs are individuals and small samples, as in our case, don't make up for a general conclusion on the matter.

The two dogs recognized the scent of *Cerambyx cerdo* larva and they could tell it apart from other scents. They felt comfortable to indicate on the scent when they physically reached it and they localised the targets with a high number of accuracy on the blind trial.

The training will continue in order to help the dogs communicate their findings on targets that are not reachable. The aim is then to use the dogs in the reintroduced localities on the main land in Sweden. By that time, we will know if the trees were successfully infested with the larva and if they developed into adult beetles of the Greater Capricorn beetle.

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