Evaluating the Feasibility of Training Scent Detection Dogs to Identify Specific Ant Species for Advancing Field Research Technology.

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Abstract

Detection dogs are an increasingly important tool in conservation. The use of trained dogs can significantly reduce effort, improve field survey accuracy, and increase the area searched compared to traditional on-ground survey methods that rely on human identification (Beebe, 2016, Bennett, 2022). Two dogs were trained to search for the Byre of the attendant Coconut ant *(Papyrius sp.)* of the cryptic and endangered Ant Blue *(Acrodipsas sp.)* butterflies. Trained using Operant conditioning techniques, the dogs demonstrated high accuracy in identifying the target scent within a controlled setting. Both dogs demonstrated a high degree of species specificity to the target odour, underscoring this method's efficacy in detecting cryptic endangered species in their natural environments. The data indicates that employing an optimised training approach can rapidly achieve high specificity (97%) and high sensitivity (90%).

Keywords: Scent Detection; Passive-indication; Operant Conditioning; Dog; Invertebrate Conservation

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Summary Statement

Detection dogs, trained using operant conditioning, significantly enhance conservation efforts by improving survey accuracy and efficiency. This study highlights their success in identifying the Byre of Papyrius ants, aiding Ant Blue butterfly conservation.

Introduction

The Small Ant Blue butterfly *(Acrodipsas myrmecophila)* is experiencing a marked decline, having vanished from seven of its eight known habitats. It faces significant threats, including potential extinction, primarily due to its reliance on the Coconut ant and the fragile state of its environment (DES, 1996). The overarching conservation goals for this species are to ascertain its current distribution, further investigate its ecological requirements, and safeguard existing populations.

Butterfly Community No.1 comprises a collection of rare and extremely rare invertebrate species that require urgent monitoring and management to prevent extinction (New, 2011, New, 1997). This community is located at the Mt Piper Conservation Reserve, Broadford, and holds particular significance as it is the sole known habitat where both the large Ant Blue butterfly *(Acrodipsas brisbanensis)* and the Small Ant Blue butterfly co-exist (Boehm, 2022).

The Small Ant Blue butterflies lay their eggs on or near Coconut ant nests. The hatched caterpillars are protected and attended by the ants. In return, the caterpillars produce a sugary substance on their bodies, which the ants consume without harming the caterpillars. This relationship is somewhat mutual; however, the butterflies are entirely dependent on this genus of ant for their survival, whereas the ants can survive independently of the butterflies (Bond, 2019, DSE, 1996).

Both the attendant Coconut ants and butterfly species are presently in decline due to land use change, clearing and other environmental issues. The small Ant Blue butterfly was deemed to have disappeared from its final refuge in Mt Piper, until Threatened Species Conservancy (TSC) discovered breeding evidence in March 2022 during volunteer-run Coconut ant surveys around the Mt Piper/Broadford region (Boehm, 2022). The substantial time commitment required from volunteers prompted us to investigate whether dogs could expedite the search process.

Using trained detection dogs, our objective was to 1) evaluate a novel method for identifying attendant ant nests associated with threatened butterfly species, and 2) to substantially search the area for the associated Coconut ants and contribute to a comprehensive taxonomic revision of the Coconut ant genus *(Papyrius sp.)* by locating additional ant nests and collecting more samples. We examine the practical considerations essential for selecting and training dogs to detect threatened species in situ, relying solely on olfactory cues and excluding visual cues.

Materials and Methods

Scent Collection

The Coconut ant Byre was collected from one of two positively identified locations within the Colin Officer Reserve in Broadford, Victoria. This site hosts a large and thriving colony of Coconut ants, whereas the second site contains a small, emerging community that was left undisturbed to avoid disruption. The Byre was collected after verification by the Threatened Species Conservancy field officers, placed into clean zip-lock bags using disposable latex gloves, and subsequently frozen for use in scent training. Given that both dogs exhibited an aversion to ants, the Byre was chosen for its significant interest to them. Photographic identification was also used to verify the ant species, including precise geographic location data, using Geosetter software and the Geo Tracker application on the mobile devices of the collectors.

Detection Dogs

The two English Pointers used in this study exhibit high drive, proven endurance, agility, and strong trainability. Their robust, innate behaviours attributed to their working heritage and breeding, were initially honed through passive-indication scent-detection training on Koala scat obtained from Melbourne Zoo. Since transitioning to the target scent of Coconut ant Byre, neither dog has been trained on any other scent. At the commencement of the new target odour training, the dogs were two and three years old, respectively. Neither of the two dogs are certified scent-detection dogs, both belong to the author, and no animals were harmed during the study.

Scent training (focus scent, controlled environment).

Training dogs to passively indicate the presence of a target odour requires the use of Operant Conditioning. The terminology associated with this system is detailed in Table 1 below. The training for this odour was conducted in three phases, informed by similar studies (Matthew, 2023, Rutter, 2021, Johnen, 2013, Roberts, 2023).

The initial phase took place indoors within a controlled environment, devoid of natural elements such as wind or humidity. The second phase transitioned to an outdoor setting, incorporating some controlled factors, including odour placement and environmental stimuli. The third phase occurred within the local surroundings, but no quantifiable data was collected from these sessions. No indications were recorded during the initial training phase. Due to the dogs' adverse reactions to the ants, which they preferred to avoid than approach, Byre was selected as the focus scent instead of deceased ants. At the commencement of each phase, the dog was presented with a sample of the target scent in a small canister with a perforated lid to familiarise them with the target odour.

Table 1. Training terminology used in the operant conditioning of dogs.

Terminology	**Explanation**
Indication	An operant conditioned response or behaviour: pointing, sitting, lying down facing the target.
Focus Target	A target location containing the scent/odour that the dog is being trained on
Alternate/Negative Target	A target location containing an odour or scent that does not match the target odour that the dog has been trained on
Control	An empty or clean container or location.
Disturbance	Anything that can be interpreted as a distraction during the training or testing: other scents; people interacting with the dog; other animal incursion to session.
Miss	A lack of indication of a positive target.
False Indication	An indication made on a negative or a control target.
Test Run	A test run involves the dog examining 10 holes on the platform, providing one opportunity to indicate the positive target. The dog is scored after each run, and the targets are replaced and rearranged.

Both dogs were trained to search for and indicate the target by sitting or lying down facing it, known as shaping behaviour. They received a food reward for correctly identifying the target scent, with the reward timing gradually increased to mimic field conditions. This reward-based training reinforced their ability to indicate the focus scent.

A platform featuring ten 8cm diameter holes, elevated 8cm from the floor to create a false bottom, was constructed to prevent the dogs from relying on visual clues. This platform with each hole numbered from one to ten (Figure 1), facilitated easier data quantification. Each training run involved the dog navigating from the first hole to the tenth hole.

Figure 1. The test board shows numbered, evenly spaced holes for odour canister placement.

Immediately before training, one of the dedicated canisters was loaded with the Coconut ant Byre and concealed in one of the 10 holes in the platform, while the remaining nine either remained empty or one hole also contained the similar ant species Byre. The dogs were guided to investigate each hole on the raised platform using the command “find” (Figure 2).

To enhance the dogs’ precision in indicating a positive target during training, a clicker was employed as a conditioned secondary reinforcer, along with a reward. If either dog falsely indicated a target, no reward was given. This method, known as negative punishment, aims to teach the dogs to indicate only on the focus target.

Figure 2. (left to right). Coconut ant Byre, scent detection dogs conduct a search, indicate a target, and receive a reward.

The dogs exhibited a keen interest in the nest material (Byre) produced by the ants during nest construction and excavation. As a result, live or dead specimens were not required during our training.

Scent training – (outdoor uncontrolled environment).

Both dogs were trained using ant Byre contained within specialised scent-training canisters, which were concealed in various outdoor environments, including nature-strips, local parks, and state-managed nature parks. The outdoor training spanned four weeks and involved diverse distraction set-ups. All sessions were conducted with the dogs equipped with harnesses, long-line, Garmin GPS collars and a Garmin GPS tracker. As the dogs demonstrated increased focus and reduced environmental distraction, the complexity and number of runs progressively increased. During these sessions, the handler concealed the positive target canisters within the designated search area, and each dog was permitted to search until a correct identification was made, irrespective of the time taken.

The active dog was instructed to “find”, with the handler accompanying it along the tracks, typically starting downwind to simulate a natural search scenario. Up to 12 scent canisters containing Coconut ant Byre were randomly hidden along these tracks for the dog to find. To minimise human interference, a QR code detailing the canisters' purpose was affixed to the bottom of each container. Upon successfully identifying a target, the dog was rewarded and then directed to “find” additional targets along the route. Due to the varying locations and diverse areas utilised in these training sessions, the data could not be quantified. Instead, it was used to predict factors that might influence a dog’s performance during field trials. The hander frequently traversed different walking paths and routes to prevent the dogs from tracking the handlers' movements rather than searching for the target itself. Although we did not collect quantitative data for outdoor training, the team completed 40 field training sessions per dog. Despite numerous distractions, both dogs consistently located the target odour.

Experiments 1 and 2. Focus odour testing (Papyrius Byre, controlled environment).

Experiments 1 and 2 were each conducted 40 times, resulting in a total of 40 positive targets and 360 negative targets each across both experiments. An assistant, wearing disposable gloves, randomised the sample location using one of ten pre-loaded canisters of Coconut ant Byre, and sanitised the platform and containers with odourless sanitising wipes between each run. During each run, the handler directed the dog to the platform, which contained ten numbered locations. One of these locations included a positive target odour. To avoid handler bias, the search team (comprising dog and handler) remained outside the test area while the targets were changed, and surfaces were cleaned. Experiment 1 was held indoors in a controlled environment, while Experiment 2 was conducted outdoors in a controlled setting.

Experiments 3 and 4. Species-specific testing (Similar species, controlled environment).

These sessions mirrored experiments 1 and 2, with the addition of a new, similar ant species to assess the dogs' ability to differentiate between the two species and accurately indicate the target species. In this study, the similar ant species was *Ochetellus sp*. We used Byre from the *Ochetellus* ant nest, as the ant emits a scent similar to that of *Papyrius* (Coconut ants) when crushed. *Ochetellus* is a fairly common close relative of *Papyrius* and is often found in forested and woodland areas, creating trails up tree trunks similar to *Papyrius (Antwiki, 2024)*. Unlike *Papyrius*, *Ochetellus* are small (2 – 3mm), black ants that appear distinctly different from *Papyrius*. Their scent is less intense, less sweetly coconut-like, and has overtones of crushed ant smell or formic acid, reminiscent of the odour associated with squashed *Iridomyrmex* (meat ants). The Byre from the *Ochetellus* species was collected, stored and used identically to the Papyrius Byre. In alignment with experiments 1 and 2, experiments 3 and 4 were conducted in controlled environments, with experiment 3 taking place indoors and experiment 4 conducted outdoors.

These experiments were conducted over three weeks, with no more than 20 runs per dog per day. The dogs were rested between groups of runs and no dog worked for more than two hours at a time. These experiments produced detailed, quantifiable results.

Field Trials (natural, uncontrolled environment).

The field trials took place within the known area for Coconut ants that coexist with the Small Ant Blue Butterfly, namely the Colin Officer Reserve, Broadford, Victoria (Figure 3). In all field test sessions, the dogs were initially presented with a sample odour to cue them to match the scent to the target odour. Conducting the searches during winter (August) minimised the necessity to avoid midday heat and mitigated the risk of snake bites. The dog/s were rested every hour or when necessary, with access to fresh water at all times. Due to the area being a popular nature trail for dog walkers and hikers, these field trials were conducted early in the day as close to first light as practicable, to attempt to keep visual and auditory distractions to a minimum. Field assistants were instructed to remain silent at all times, walking behind the handler-dog team to record information and keep the team on track. During these runs, the dogs were equipped for outdoor training, with the addition of a TPU muzzle to avoid potential harm to wildlife encountered during the tests.

Figure 3. Field search location, Colin Officer Flora Reserve, Broadford. Victoria.

Once the dog/s were able to locate the target odour successfully within the 40 controlled outdoor test runs, the dogs were individually trialled on known locations in the field. The study area is approximately 13.7 hectares (Gov, 2024) and is comprised of scrubland with several footpaths crisscrossing it. When possible, wind direction influenced the start of the searches, allowing the dogs to work against the wind and any scent blown towards the dog/handler team. This aims to amplify detection probability, thereby optimising efficiency in these search operations. On average, each dog worked a search session of 2 hours or less. The team did not deviate from the paths unless the dog picked up on a target scent. Both dogs were trained to indicate by sitting when they located the target. The dogs were only rewarded if the target was correctly identified by the handler. If identification could not be confirmed, the search then continued with no reward, the dog being asked to “find” once more.

Data Analysis

In Experiment 1 of the testing, a correct indication was documented when the dog alerted the handler to a positive target. Conversely, a miss was recorded if the positive target was not identified, and a false indication was noted if the dog alerted to a negative target location or a distraction. Data was meticulously recorded using a digital form on personal mobile devices, producing a detailed, time-stamped spreadsheet. These results were used to calculate sensitivity, defined as the ratio of correct indications to the total number of positive targets. To prevent naming confusion with similar headings, each category was distinctly renamed. Sensitivity was calculated as follows: **Eqn1**: ***A****ccuracy* = ***c****orrect* ***i****ndications/****p****ositive* ***t****argets.* $\boldsymbol{A = \frac{ci}{pt}}$

Misses were quantified by comparing the number of correct indications to the total number of positive targets. This metric served as an indicator of the dogs’ reliability in detecting the target odour and was calculated by incorporating the number of incorrect indications into the equation. Thus, efficacy was calculated as follows: **Eqn2**: ***E****ffectiveness* = ***c****orrect* ***i****ndications/ (****p****ositive* ***t****argets* + ***i****ncorrect* ***I****ndications).*$\boldsymbol{E = \frac{ci}{pt+iI}}$

Specificity to the focus target odour, amidst negative targets, disturbances, and distractions, assessed the dog’s ability to precisely indicate the target without yielding to distractions. Specificity was calculated as follows*:* **Eqn3***:* ***P****recision* = ***c****orrect* ***i****ndications/ (****c****orrect* ***i****ndications* + ***i****ncorrect* ***I****ndications).*$\boldsymbol{P = \frac{ci}{ci+iI}}$

Results

Experiment 1 and 2 testing focus target only, controlled environment.

This phase encompassed four distinct experimental designs, each comprising up to 400 targets, with 40 being positive and 360 negatives. In experimental designs 3 and 4, while there were still 40 positive targets, a similar species odour was introduced per run, leaving 320 empty locations in the board. Both correct and incorrect indication results for each dog across all experiments are summed in Figure 4.

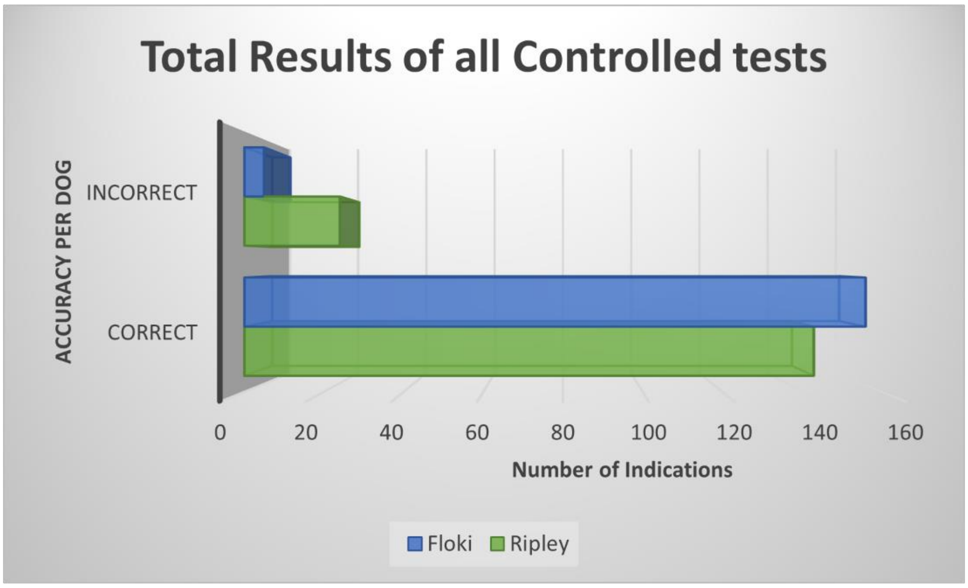

Figure 4. Number of correct and incorrect indications for each dog combined for all controlled tests

Below, figure 5 illustrates the sensitivity, or accuracy of both dogs. The highest accuracy for both dogs was recorded during Controlled Experiment 1, conducted in an environment devoid of distracting influences. In this experiment, the dog Floki achieved an accuracy of 96%, while the dog Ripley achieved 90%.

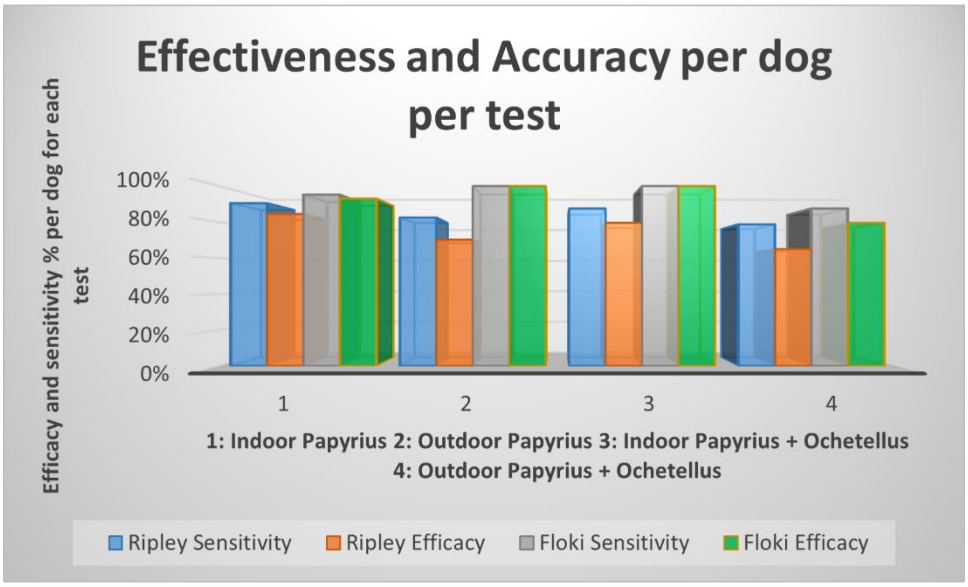

Figure 5. Effectiveness and accuracy percentages achieved by each dog across the different experimental designs.

Experiment 3 and 4 testing for focus target, controlled environment.

In experimental designs 3 and 4, the inclusion of scent material from a different ant species required the dogs to differentiate between the two species. Floki's performance was consistently above 90%, with an average precision for the target odour of 97% and an average effectiveness of 93%. Ripley's indoor precision results were all above 97%, with an average specificity (or precision) of 86% and an average effectiveness of 74%. Specificity, or species-specific precision, was determined by calculating the number of correctly identified positive targets by the dog (Figure 6).

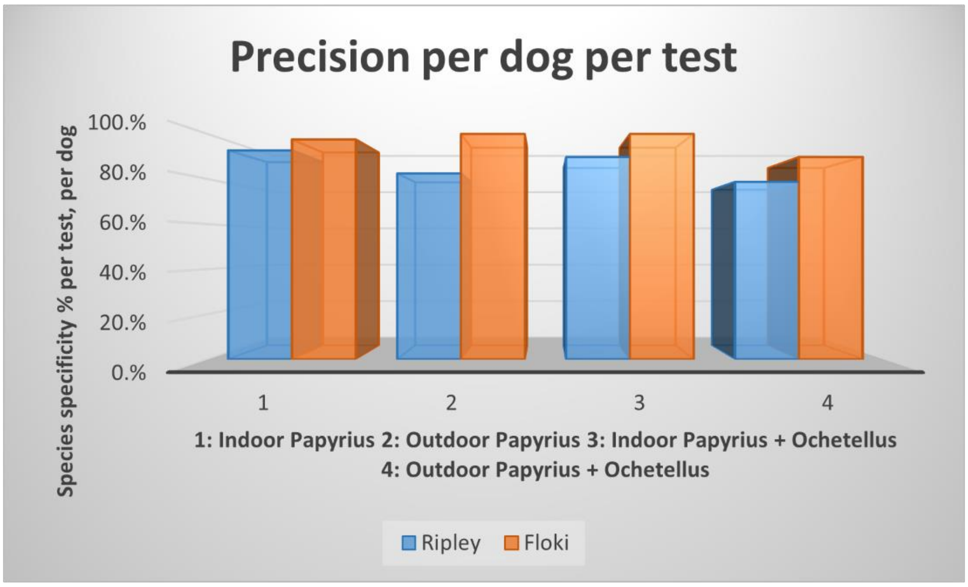

Figure 6. Species specificity results for both dogs for each test design.

Across all four experiments, Floki maintained an overall accuracy of 96%, whereas Ripley's overall accuracy was 86% (Figure 7).

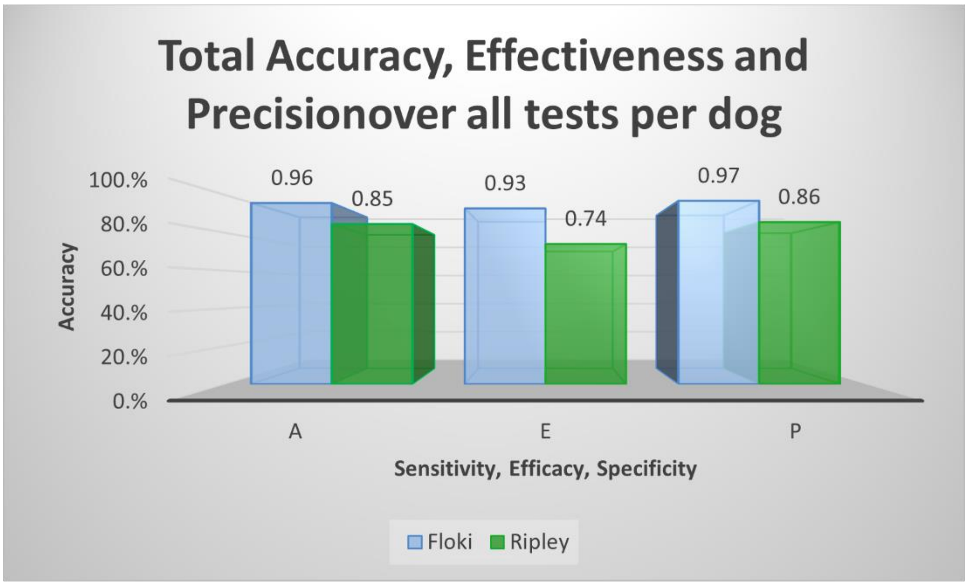

Figure 7. Summed percentages of total accuracy, effectiveness and precision across all tests for both dogs, providing an overall assessment of this technology's suitability.

Scent detection - field trials

The trained dogs were individually deployed at the Colin Officer reserve to investigate whether there was a greater presence of the Coconut ant. The dogs indicated multiple times during the 10 deployments. Due to the scale of colonisation at one location, it is possible that the dogs indicated on the same colony more than once. No incorrect indications were observed during field trials, although there were large stretches in which no indications occurred. Additionally, during each deployment, the dogs became increasingly distracted by other stimuli, exhibiting high alertness and reluctance to indicate correctly (sit and wait).

Quantifiable data were not collected for these trials due to the search area containing only two known *Papyrius* sites, making randomisation of locations infeasible.

Discussion

Having successfully shown their complex olfactory capabilities to successfully locate the Coconut ant nest material, both in controlled and natural settings, this methodology shows promise for boosting conservation research in endangered species. We demonstrate it is possible to train a scent dog to locate the host of a cryptic and rare butterfly in a natural environment using non-invasive means and no-harm practices. The success of this training methodology became evident during all test sessions when both dogs were able to detect over 85% of positive targets. This result could be particularly valuable for detecting cryptic and elusive invertebrates where, the next challenge will be to locate the caterpillars (Braby, 2021), thereby further refining search outcomes.

The potential for the use of detection dogs in conservation science is growing exponentially. Therefore the handler must consider the effectiveness of the dog (De Matteo, 2019), acknowledging the uncertainty in predicting specificity in the face of related species in the greater environment, as species in the same genera share a greater number of signature elements, such as odour, than those in separate genera (Hurt, 2009). It has been found in some studies that if a focus scent is too similar or too contradicting, that can potentially lead the dog to a false indication, or no indication at all (Lit, 2006). Because the dogs were not trained in the geographic environment where the species is known to exist, these environmental factors could likely cause increased distractions and therefore negatively affect the dogs' performances, therefore these factors should be considered before and during ex-situ training, and every effort should be made to train the dog in the environment in which it will eventually be deployed.

The use of trained scent dogs could prove to be an invaluable and faster means of confirming the presence and distribution of the focus species in the research area. The olfactory sensitivities of such trained dogs have helped councils, wildlife conservationists and land management ecologists to locate not only protected species but also invasive and feral pest species (Moser, 2020, Wasser, 2004, Braun, 2013).

The results of the controlled experiments demonstrated that the accuracy, or sensitivity of the dogs' remained unaffected by the introduction of a secondary species odour. The Coconut ant emits a distinct coconut scent when crushed, whereas the closely related *Ochetellus* species has a more citrusy odour. However, as no ants were harmed during these experimental setups or training sessions, and only the nest material was used for odour recognition, it is possible that the dogs were identifying the species of tree utilised by the ants for their colonies. Further experiments may be required to identify a more distinctly recognizable scent if dogs are to be employed in presence-absence surveys for this species in the future. This could provide crucial information regarding the specific tissue type needed to train dogs to detect these rare and cryptic species effectively. An alternative hypothesis suggests that storing and preserving the ant byre of both species in a freezer could cause the scent to weaken over time and become more similar, thereby making it more challenging for the dogs to distinguish between them. However, this argument is invalid, as neither dog confused the *Ochetellus* ant Byre with the target odour during any of the controlled experiments, demonstrating a high sensitivity to the Coconut ant Byre.

Effective training for the dog and the handler is fundamental, focusing on the handler's natural responses (Lit, 2006). On completion of training, the testing should replicate the field environment as closely as possible (De Matteo, 2019). However, a scent-work field-study study should not be designed to fit a specific detection dog, nor should one choose a dog based solely on its tight bond with the handler. Rather, the study design should be utilised to assess the suitability of employing a detection dog and to subsequently guide the selection of an appropriate dog, diverse training samples and handler (Jamieson, 2018). Professional training can help to ensure that this extraordinary conservation method retains the high accuracy it originally had in military, police, and emergency rescue applications (Jamieson, 2018).

Building on these findings, the next step would be to expand the search across the Ant Blue's range to locate additional Coconut ants. These ant nests can then be assessed for breeding evidence of the threatened butterfly, potentially mitigating the risk of this species' extirpation.

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Competing Interest: While the Threatened Species Conservancy provided access to the field location for the collection of scent material, and the researcher's supervisor was an employee of the Conservancy, no financial benefit, nor content control occurred during this research.

The dogs used in this research belong to and were trained by the primary author.

Statement: During the preparation of this work the author(s) used a web-based AI tool to condense lengthy original text. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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Figure Legend

Figure 1. The numbered test board with evenly spaced holes for odour canister placement.

Figure 2. (left to right). Coconut ant Byre from fied site, scent detection dogs conduct a search, indicate a target, and receive a reward.

Figure 3. Field search location, Colin Officer Flora Reserve, Broadford. Victoria.

Figure 4. Number of correct and incorrect indications for each dog combined for all controlled tests

Figure 5. Effectiveness and accuracy percentages achieved by each dog across the different experimental designs.

Figure 6. Species specificity results for both dogs for each test design

Figure 7. Summed percentages of total accuracy, effectiveness and precision across all tests for both dogs, providing an overall assessment of this technology's suitability.

Figure S1. Geosetter software used for geotagging photographs of ants in the field.

Figure S2. Hand-held GPS devices used in tracking and geolocating indications in the field.

Figure S3. Research assistants setting up experiments 1 through 4.

Figure S4. Scent board in outdoor controlled setting (left) and 12. Research Assistant with the scent detection dogs (right).

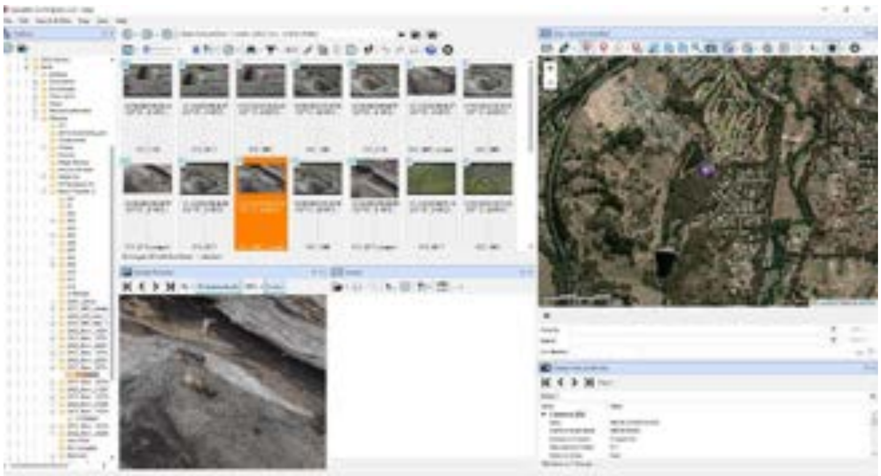
Figure S5. Geo Tracker (V.5.3.4.3912) app (android) for logging photographic locations for Ochetellus sp.

Appendix

Table S1. Equipment used to train the 2 dogs.

Table S2. Coconut ant nest site 1. And emerging site 2, as indicated by the dogs in Broadford, Victoria

Table S3. The dogs indicating in the field at Colin Officer Flora Reserve.



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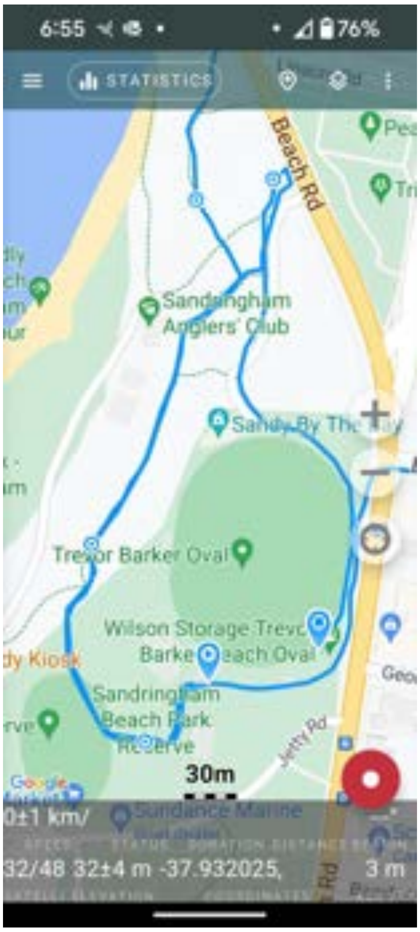

Figure S6. Geo Tracker (V.5.3.4.3912) app (android) for logging photographic locations for Ochetellus sp.

Table 4. Graphical representation of random Byre placement for experiments 1 through 4 from data collection spreadsheet.

Table S5. QR codes were used during experiments 1 through 4 for data collection.