Canine Research

Training dogs to detect invasive alien species in Japan: Discrimination of reptile odor

Megumi Fukuzawa*, Marina Sasahara

Nihon University, College of Bioresource Sciences, Fujisawa-shi, Kanagawa, Japan

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ABSTRACT

The use of dogs for detecting invasive animal species has increased in Japan. Damage to endemic species by the Carolina anole (*Anolis carolinensis*) is expanding in the Ogasawara Islands. We hypothesized that dogs trained to recognize the anole's odor would be useful in detection work. A healthy adult female German shepherd was trained to discriminate anole odors by using a cloth onto which we had transferred frozen body odor or frozen excrement/urine odor, along with an odorless cloth. After achieving 100% accuracy, she was trained to discriminate between body odor and excrement/urine odor of the same anole. Then the dog was trained in a three-way test to discriminate among native Ogasawara reptiles and the anole. The dog achieved 100% accuracy in training for body odor in 27 sessions over 14 days and for excrement/urine odor in 4 sessions over 2 days. The mean correct search time differed significantly between odors in training ($P < 0.05$), but not in the discrimination test ($P = 0.71$). The rates of the correct response in the two-way discrimination test were high (body odor, 90%; excrement/urine odor, 96%). Correction rate in the three-way test was >90%. Discrimination between transferred odor and the odorless sample in training was relatively easy. However, discrimination between odors of the same anole and between odors of the native reptiles and the Carolina anole was harder.

Introduction

The Carolina anole (*Anolis carolinensis*) is a lizard of Southeast US origin, but has become established in the Ogasawara Islands, in Okinawa and on Zamami Island in Japan. Nucleotide sequence analysis showed that the invader originated in the western part of the Gulf Coast and inland areas of the United States, and that some of the anoles on Okinawa were not introduced via the Ogasawara Islands (Suzuki-Ohno et al., 2017). The native insect community in Ogasawara and Okinawa has declined greatly in all areas owing to predation by the anole. The Japanese Ministry of the Environment, therefore, listed the Carolina anole as an invasive alien species in Japan in June 2005, barring its spread to new islands and setting natural regeneration zones in remaining habitats of native insects. It has also promoted a project to eliminate the anole from these areas and to improve the number of insect communities there. The anole is controlled by setting polypropylene adhesive traps around tree trunks to capture them, and by the erection of barrier fences and electric fences. Adhesive traps are effective except where numbers are low, and so do not allow us to judge whether a population has been eliminated or not.

The Ogasawara Islands were registered as a World Heritage Site in 2011 because they provide evidence of the different stages of the evolution of endemic species on oceanic islands, and the evolution of marine species into terrestrial species. These islands are also known as the Galapagos of the Orient, and to conserve rare ecosystems and rare wild animals that live only on these islands, management of invasive alien species is an important issue. The Ogasawara Islands are located in the western Pacific Ocean, along the Ogasawara Ridge, roughly 1,000 km from Tokyo, approximately 25 hours by boat. Therefore, it is necessary to test whether a detection dog trained to detect the anole in the main Japanese Archipelago can demonstrate its capability on site after moving by boat.

Dogs can detect very faint odors, and are routinely used in search works. Dogs are also used for exploring conservation areas. For example, Cablk and Heaton (2006) used dogs to detect desert tortoise (*Gopherus agassizii*), and they concluded that dogs are capable of detecting tortoises in burrows under a range of

environmental conditions. Mitani et al. (2014) used dogs to detect alien mongooses (*Herpestes auropunctatus*) in Japan. Becker et al. (2017) used dogs to detect cheetahs (*Acinonyx jubatus*) across large distances in Zambia. To examine the effectiveness of dogs in searching for the Carolina anole in Japan, we trained a dog to recognize the odor of the anole and tested its ability to discriminate between the anole's body odor and its excrement/urine odor, and between the body odors of native Ogasawara Island reptiles and the anole. If dogs can discriminate between these odors, they may be able to help locate and capture anoles without harming native species.

Materials and methods

Animal

We trained a healthy adult female German shepherd named Fico (28 months, 25.8 kg) with the agreement and participation of the owner. Fico has received obedience and tracking training as a police dog from six months old, thus has already learned to point at target odors with "lay down" posture. Balls are usually used as reinforcer in her daily training. Fico was kept in a familiar kennel at the university during the day, where she was allowed to contact with the outdoors and with humans at all times, and returned home at night.

Target odors

We used 10 male Carolina anoles (caught on Chichijima, Ogasawara Islands, in August 2014) and their excrement/urine, 5 male mourning geckos (*Lepidodactylus lugubris*), 5 house geckos (*Hemidactylus frenatus*), and 5 Ogasawara lizards (*Cryptoblepharus nigropunctatus*). The anoles were kept at a university facility that has permission to rear alien organisms, and were killed after observations by pentobarbital overdose (approval AP16B001). Each anole and its excreta (3-5 lumps) were placed in separate containers and kept in a freezer (-30.0°C). The other three species were caught on Chichijima in August 2017 and were also kept in a freezer. Just before each training session or test, the gloved handler rubbed a white odorless cloth (100% cotton) against one frozen sample for about 5 seconds to transfer the odor using stainless steel tweezers, to avoid the odor contamination. Gloves and tweezers were exchanged every time. The cloth (or an odorless control cloth) was placed in a 100-mL polypropylene bottle with multiple holes (3-5 mm diam.) in it so that the dog could not contact the cloth directly (Figure 1). Bottles were placed on stainless steel dishes 60 cm apart (50 cm in three-way test) on a stainless steel table (88 cm long $\times$ 19 cm deep $\times$ 17 cm high; Figure 2).

Procedures

All training trials and tests were carried out in the same room (210 cm $\times$ 345 cm). The room held the stainless steel table on which the odor bottles were set. The handler sat 2 m from the dog's start position. To record the dog's search behavior, we set a video camera (DCR-SR87; Sony, Tokyo, Japan) before the table and another behind the table. The room temperature was controlled at around 20.0°C (mean, 18.6 $\pm$ 1.5°C). For both the training trials and the discrimination tests, the dog was brought from her kennel to the room.

Stage 1 of training used the body odor cloth (positive stimulus) and an odorless cloth (control). Stage 2 used the excrement/urine odor cloth and an odorless cloth. At the beginning of each trial, the handler presented Fico with a bottle containing the odor cloth to smell for up to 60 seconds. When the handler judged that Fico recognized the odor (e.g., Fico diverted her face from its odor source and tried to head to the front table), she told Fico to "search" and

Figure 1. White cloths placed within perforated polypropylene bottles.

released her. When Fico recognized the odor in the bottle on the table, she laid down in front of it. Fico was given 180 seconds to choose. When she selected the correct bottle, she received a food reward. When she selected the incorrect bottle, the trainer ignored her for 3 minutes. Each session consisted of 20 such trials. Fico was allowed for short rests both between and during sessions. Two sessions were performed each day, 4 or 5 days a week. The trainer collected data from the recorded videotapes, including the time Fico took to select the correct scent (to a precision of 0.01 seconds). Each training stage required Fico to have a 100% success rate in two sequential sessions. When she reached the criterion for both stages, the two-way alternative discrimination test was started.

Each block of testing used two confirmative trials (the same conditions as during training) both before and after five discriminative trials, in which the handler presented Fico with either a body odor cloth or an excrement/urine odor cloth and then released her to select the matching odor. Thus, the confirmative trials consisted of 40 of each odor (80 trials total), and discriminative trials consisted of 50 of each odor (100 trials total). In all trials, the type of odor presented was random, and the handler did not know which bottle held the correct odor.

After the 2-way alternative discrimination test, we ran a 3-way alternative discrimination test, in which 3 reptile odor cloths were presented in 1 of 4 patterns: pattern A—Ogasawara lizard, mourning gecko, Carolina anole; pattern B—Ogasawara lizard,

Figure 2. Placement of bottles on the stainless steel table.

house gecko, Carolina anole; pattern C—mourning gecko, house gecko, Carolina anole; pattern D—Ogasawara lizard, mourning gecko, house gecko. In all patterns, Fico was required to identify the anole body odor, or return to the handler in the case of pattern D. Patterns A to C were randomly performed 3 times, and pattern D was performed at the 10th trial. These 10 trials constituted 1 session, and 10 sessions were performed (patterns A-C, 30 trials each; pattern D, 10 trials).

Statistical analysis

The number of sessions required to reach the criterion for each training stage was recorded. Differences in the numbers of sessions required for Fico to reach each odor training stage criterion and between the average search times for correct and incorrect choices in each session were compared by Mann–Whitney U-test. The effect of session on the average search time of Fico's correct and incorrect choices in each training stage was examined by Kruskal–Wallis test. When significant effects were found, we used a *post hoc* Steel–Dwass test for pairwise comparison of the means of the search times.

In the two-way discriminative trials, differences in average search times of correct or incorrect choices were compared both between odors and within each odor by Mann–Whitney U-test. Differences between the correct-response search times of the confirmative trial and of the following discriminative trial and between the correct-response search times of the discriminative trial and the following confirmative trial were compared by Wilcoxon's signed rank test. In the three-way discrimination test, differences in the search times of correct or incorrect choices among patterns A-C were compared by Kruskal–Wallis test. Differences between search times of patterns A-C and that of pattern D and differences between the search times of anole odor discrimination of pattern D and that after experiencing pattern D were examined by Wilcoxon's signed rank test.

Results

Training

Number of sessions to reach criterion

The total number of sessions for the body odor task (27 in 14 days) differed significantly from that for the excrement/urine odor task (4 sessions in 2 days) (Mann–Whitney U-test, $U = 9.5$, $P \leq 0.01$).

Search time

During the body odor training sessions, both the search time when Fico selected the positive body odor bottle ($\chi^2 = 82.96$, $P < 0.001$) and when she selected the odorless one ($\chi^2 = 86.39$, $P < 0.001$) had significant differences among sessions. When she selected the correct bottle, the mean search time was 7.81 ± 5.47 seconds, and search times of sessions 11, 13, and 14 were significantly slower than those of sessions 4, 17, 19, and 21 (Steel–Dwass test, $P < 0.05$; Figure 3). When she selected the incorrect bottle, the mean search time was 8.75 ± 6.07 seconds, and the search time of session 13 was significantly slower than those of sessions 4, 5, 9, 17, 19, 21, 24, and 25 ($P < 0.05$; Figure 4).

During the excrement/urine odor training sessions, the mean search time when Fico selected the positive stimulus was 8.52 ± 4.78 seconds, and that when she selected incorrect stimulus was 7.39 ± 2.21 seconds. On the other hand, there were no differences in the search times of either the positive odor bottle ($\chi^2 = 3.87$, $P = 0.28$) or of the odorless one ($\chi^2 = 0.33$, $P = 0.56$).

Two-way alternative detection test

In the test in which the dog had to select the odor presented by the handler (body odor or excrement/urine odor), the rate of correct selection was $\geq 90\%$. There was no significant difference between the search times when Fico made the correct choice for body odor and for excrement/urine odor ($P = 0.71$), or the incorrect choice ($P = 1.00$). In addition, no significant difference was found in the search times of correct and incorrect response, respectively (body odor, $P = 0.26$; excrement/urine odor, $P = 0.47$) (Table 1).

During the alternative test session, a confirmation test (body or excrement/urine odor and odorless cloth) was inserted; the rate of the correct choice was 98% for both body odor and excrement/urine odor. The search time when the dog made the correct choice was 15.22 ± 9.48 seconds for body odor and 14.53 ± 8.72 seconds for excrement/urine odor. The dog made an incorrect response only once in each confirmation test (15.15 and 35.56 seconds, respectively).

There was no significant difference between the search time of the initial confirmation test and that of the alternative test ($P = 0.84$), or between the search time of the alternative test and that of the following confirmation test ($P = 0.40$).

The mean search time of the training sessions in which the training criterion was reached (body odor, sessions 26 and 27; excrement/urine odor, sessions 3 and 4) was significantly shorter than that in the confirmation trials for both body odor (9.62 ± 8.15
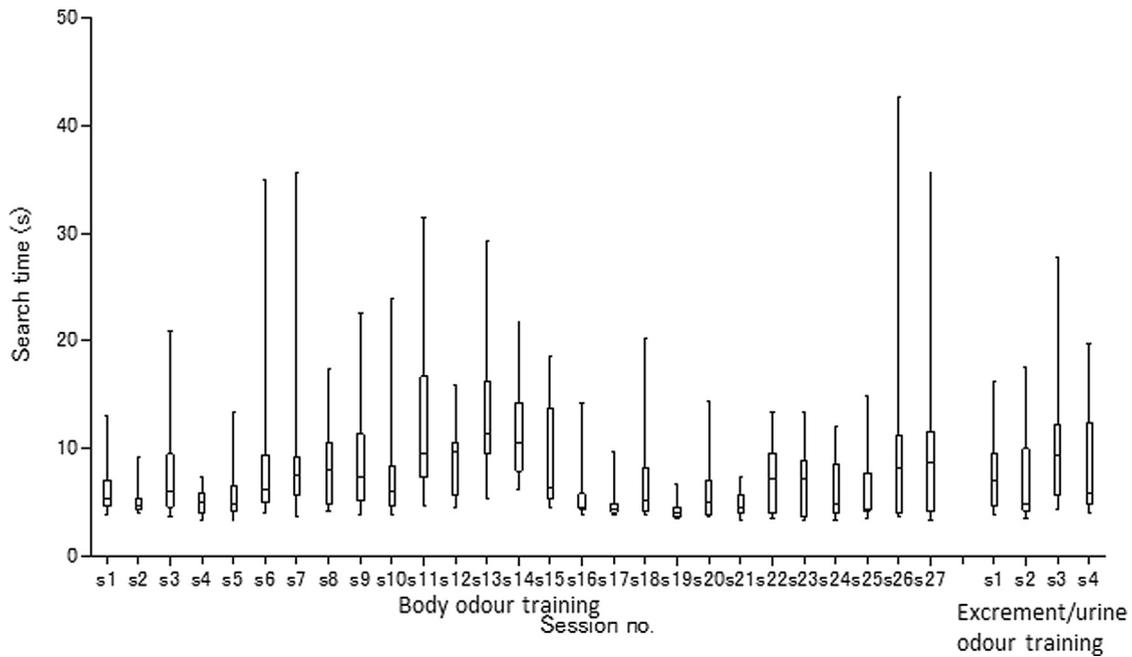
Figure 3. Search time when the dog selected the positive odor bottle during all training sessions.

vs. 15.22 ± 9.48 seconds; $P < 0.001$) and excrement/urine odor (9.28 ± 5.31 vs. 15.78 ± 6.48 seconds; $P < 0.001$).

Three-way alternative discrimination test

The rates of correct choices in patterns A-C were not significantly different (A 100%, B 97%, C 93%), nor were the mean times for correct detection (A 22.34 ± 13.31 seconds, B 18.36 ± 8.76 seconds, C 21.04 ± 12.19 seconds; $\chi^2 = 1.34$, $P = 0.51$). The rate of correct response in pattern D was 80%. In addition, there was no significant difference between the mean times for correct detection of patterns A-C (21.97 ± 13.03 seconds) and of pattern D (21.63 ± 9.62 seconds;

$U = 335$, $P = 0.65$). The search time for correct discrimination of the first anole odor after presentation of pattern D (18.74 ± 6.96 seconds) was significantly less than the pattern D average search time (29.98 ± 18.65 seconds) just before these trials ($W = 39$, $P = 0.058$).

Discussion

With 2 weeks of indoor training, the dog could correctly discriminate the Carolina anole odor ≥90% of the time. By contrast, it took >15 months, with 300 sessions of 10 minutes each, to train dogs to detect estrus in dairy cows (Johnen et al., 2015). With 10 days' training, dogs could detect synthetic molecules with an
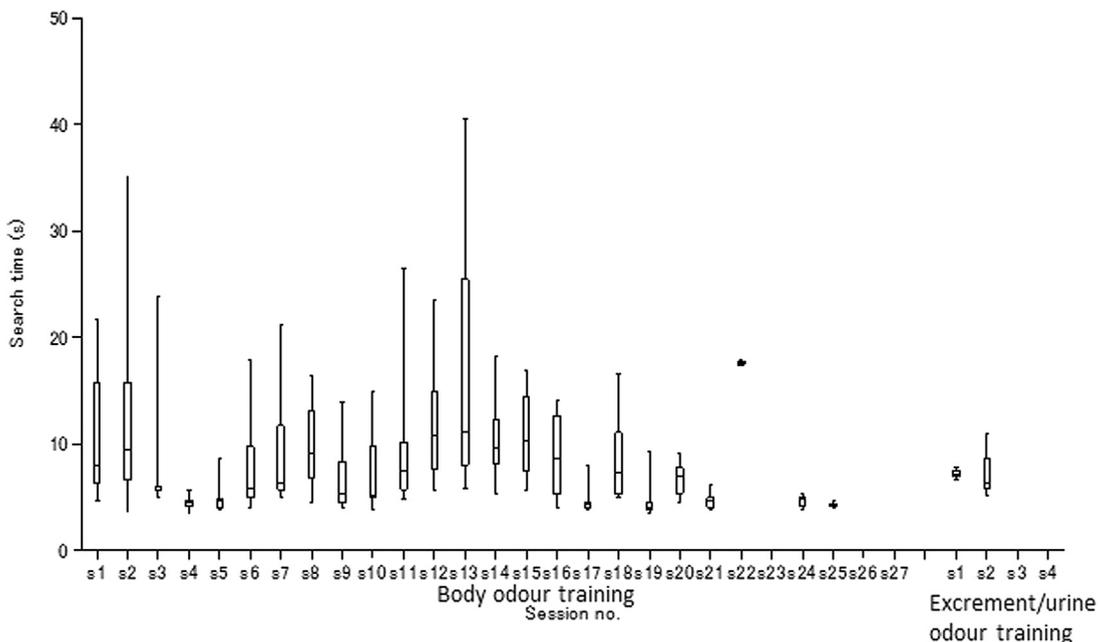

Figure 4. Search time when the dog selected the odorless bottle during all training sessions.

Table 1
The rate of correct/incorrect selection and the search times during two-way alternative detection test

	Body odor		Excretion odor	
	Percentage	Response time (s)	Percentage	Response time (s)
Correct	90% (45/50)	19.03 ± 14.56	96% (48/50)	15.78 ± 6.48
Incorrect	10% (5/50)	12.40 ± 5.20	4% (2/50)	12.44 ± 8.70

accuracy of only 69% (Fischer-Tenhagen et al., 2015). But it took only 2-3 weeks to train dogs to distinguish breath samples of patients with lung and breast cancer from those of healthy controls (McCulloch et al., 2006). These studies suggest that the accuracy of a detection task is affected by the target odor quality and intensity, yet training for only 2 weeks in 30 sessions achieved rates of correct discrimination of anole body and excrement/urine odors of 90% and 96%, respectively. It is possible that odors are affected by freezing and storage. However, Bueno et al. (2013) found that freezing had no effect on the chemical composition or odor (as perceived by humans) of lamb. In our study, the dog was able to recognize body odor and excrement/urine odor of frozen anoles with short period; therefore, the frozen body odor and the biological odor were considered qualitatively different. The reason that the number of sessions required for excrement/urine odor training was less than the number of sessions required for body odor training is indicated to be that the dog recognized the odor discrimination methodology during the body odor training period. Thus, the sensorial quality differences of the anole odor should be examined in the future.

In the field survey to detect anole, we will be likely to use the target anole excrement/urine. Currently, trap capture is effective in areas where anole population density is high. As native species are also caught in such traps, alternative ways are being considered. If dogs can reliably detect anole odor, it can be expected to be one of effective tools for helping locate evidence of anoles. On the other hand, when detecting for an anole in an area with low population density, a trap is set around the point where the dog responded to their odor. If dogs can discriminate odors by short training period and their success rate can keep high, this might lead not only to reduce the number of native species threatened by traps, but also the number of set traps and cost. Further investigations will need to consider dog breed, age, and motivation.

Dogs proved useful in determining the presence of mongoose scats on Okinawa (Fukuhara et al., 2010). Both Okinawa and the Ogasawara Islands lie in the subtropics. On Okinawa, the dogs were not hindered by distances of up to 4 km in the warm humid conditions. During our anole odor training indoors, the air conditioning was stopped and the relative humidity was 72.5% ± 9.6%. Despite differences between indoors and outdoors, such training may be useful, because this humidity range lies within the range recorded in Okinawa (55%-86%; Fukuhara et al., 2010). However, mongooses are terrestrial, whereas anoles are arboreal, so sniffer dogs must keep their heads raised, which may affect their motivation and detection range. It will be necessary to evaluate these factors.

When several mongoose scats (positive point) or blanks (negative point) are presented at 50 m intervals, dogs will investigate almost all scats (≥98%) before giving the trained alert for each scat. In our discrimination test, when we presented the anole odors at 60-cm (two-way test) or 50-cm (three-way test) intervals, the dog was able to discriminate the odors. Environmental factors such as temperature, humidity, and wind speed are likely to strongly influence dogs' ability to discriminate odors. Therefore, it will be necessary to evaluate

discrimination by varying the presentation distance of the target odor, the number of target odors, and the environment. It may also be necessary to assess the ability of dogs to detect anoles in areas with high and low densities. Future tests should also use live specimens.

It was clear that the dog could discriminate the body odors of the four reptile species living in the Ogasawara Islands with a success rate of 93%-100%. Dogs can distinguish the scents of their handlers' hands from those of strangers (Brisbin and Austad, 1991), although not between the odors of human twins identical in both genetic relatedness and environmental factors (Hepper, 1988). Even when zero choice trials were used (i.e., pattern D), the dog's correct search times for patterns A, B, and C were shorter than that for pattern D (the dog was not misled).

Conclusion

The dog was engaged in detection tasks even if trials without positive odor were interposed. It became clear that dogs can distinguish reptile species' odors living in the Ogasawara Islands and can detect for it correctly.

Acknowledgments

Ethical considerations

The Nihon University Ethics committee approved the study (EXC17B002).

Conflict of interest

The authors declare no conflict of interest.

References

Becker, M.S., Durant, S.M., Watson, F.G.R., Parker, M., Gottelli, D., M'soka, J., Droge, E., Nyirenda, M., Schuette, P., Dunkley, S., Brummer, R., 2017. Using dogs to find cats: detection dogs as a survey method for wide-ranging cheetah. J. Zool. 302, 184—192.

Brisbin Jr., I.L., Austad, S.N., 1991. Testing the individual odour theory of canine olfaction. Anim. Behav. 42, 63—69.

Bueno, M., Resconi, C.V., Mar Campo, M., Cacho, J., Ferreira, V., Escudero, A., 2013. Effect of freezing method and frozen strage duration on odor-active compounds and sensory perception of lamb. Food Res. Int. 54, 772—780.

Cablk, M.E., Heaton, J.S., 2006. Accuracy and reliability of dogs in surveying for desert tortoise (Goperus agassizii). Ecol. Appl. 16, 1926—1935.

Fischer-Tenhagen, C., Johnen, D., Le Danvic, C., Gatien, J., Salvetti, P., Tenhagen, B.A., Heuwieser, W., 2015. Validation of bovine oestrous-specific synthetic molecules with trained scent dogs; similarities between natural and synthetic oestrous smell. Reprod. Domest. Anim. 50, 7—12.

Fukuhara, R., Yamaguchi, T., Ukuta, H., Roy, S., Tanaka, J., Ogura, G., 2010. Development and introduction of detection dogs in surveying for scats of small Indian mongoose as invasive alien species. J. Vet. Behav.: Clin. Appl. Res. 5, 101—111.

Hepper, P.G., 1988. The discrimination of human odour by the dog. Perception 17, 549—554.

Johnen, D., Heuwieser, W., Fischer-Enhagen, C., 2015. How to train a dog to detect cows in heat — training and success. Appl. Anim. Behav. Sci. 171, 39—46.

McCulloch, M., Jezierski, T., Broffman, M., Hubbard, A., Turner, K., Janecki, T., 2006. Diagnostic accuracy of canine scent detection in early- and late- stage lung and breast cancers. Integr. Cancer Ther. 5, 30—39.

Mitani, N., Morosawa, T., Yamashita, R., Kioka, M., Goto, Y., Hashimoto, T., Kitaura, K., Yamada, F., Abe, S., Ishikawa, T., 2014. An evaluation of detection dogs for alien mongoose control on Amami-oshima Island. Wildl. Hum. Soc. 2, 11—22.

Suzuki-Ohno, Y., Morita, K., Nagata, N., Mori, H., Abe, S., Makino, T., Kawata, M., 2017. Factors restricting the range expansion of the invasive green anole Anolis carolinensis on Okinawa Island, Japan. Ecol. Evol. 7, 4357—4366.