Supplementary Material

Ratting on wildlife crime: Training African giant pouched rats to detect illegally smuggled wildlife

Isabelle Szott[1]*[†], E. Kate Webb[1,2,3][†], Said Mshana[1], Anthony Mshigeni[1], Walter Magesa[1], Alexander Iyungu[1], Miraji Saidi[1], Dian Van de Laak[1], Miriam Schneider[1], Ashleigh Dore[4], Kirsty Brebner[4], Harriet Davies-Mostert[4], Christophe Cox[1], & Cynthia D. Fast[1,5,6]

* Correspondence:
Isabelle Szott
szott.izzy@gmail.com

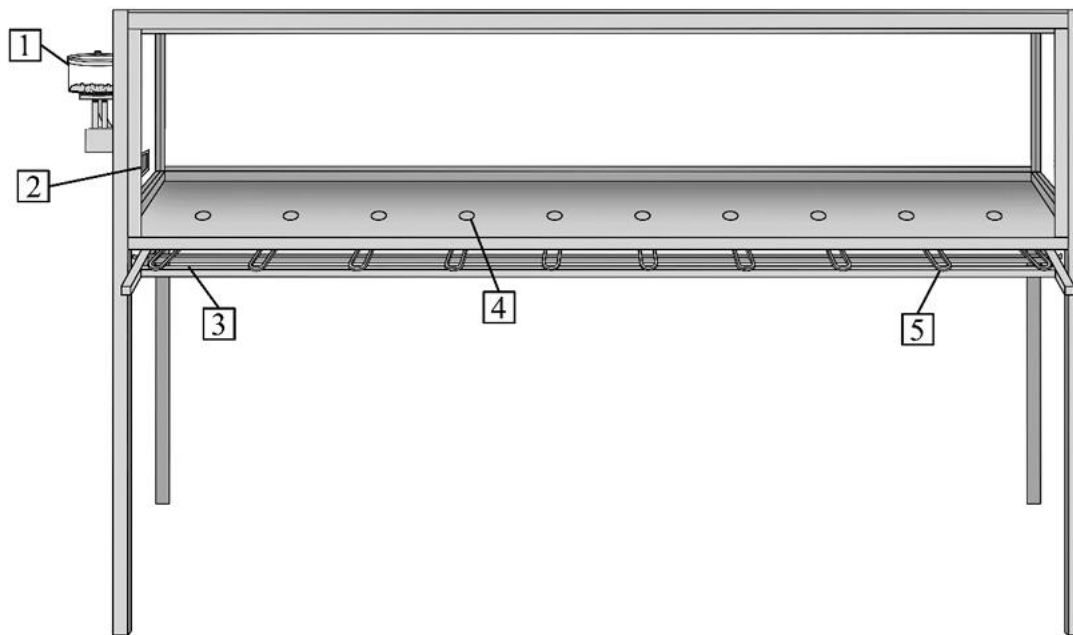## 1 Overview of training apparatus and raining timeframes

Figure S1. The semi-automated line cage is connected to a computer running specialized software. Trainers must manually open the holes along the bottom. The indication threshold is recorded when the rat inserts its nose into a hole and "breaks" the infrared photobeam. Reinforcement (in the form of small round pellets) can be provided in the cage via the automated food hopper (1) when certain programmed criteria are met (e.g., indication threshold). A plexiglass divider can be inserted so that less than 10 holes are accessible.

Components include (1) pellet dispenser and (2) magazine, (3) odor cassette, (4) 10 odor sampling holes, and (5) metal plates that can be manually moved to cover or expose the odor holes.

Supplementary Table 1. Overview of exact dates (chronologically) and number of sessions for each training phase presented in the main manuscript and described within the supplementary materials.

Training /Target material and presented where	Phase	Start date	End date	Sessions
Pangolin and Wood, main text	Indication	05/12/2017	08/01/2018	20
	30-sample discrimination	09/01/2018	02/05/2018	66
	50-sample discrimination	03/05/2018	02/07/2018	36
	100-sample discrimination	04/07/2018	10/10/2018	50
	Generalization Test	11/10/2018	22/10/2018	9
3-week break				
Ratios, supplementary material	Wood	19/11/2018	26/02/2019	55
	Wood Ratio Test	27/02/2019	08/03/2019	8
2.5-week break				
	Pangolin	28/03/2019	21/06/2019	54
	Pangolin Ratio Test	25/06/2019	04/07/2019	8
Approximate 7-month break				
Re-train Pang/Wood, supplementary material	Indication	10/02/2020	20/02/2020	8
	30-sample discrimination	21/02/2020	02/03/202	7
	100-sample discrimination	03/03/2020	27/03/2020	12
Increased non-targets, supplementary material	Training	30/03/2020	17/08/2020	77
Pang/Wood mixtures, main text	Baseline	18/08/2020	27/08/2020	4
	Familiar mix	31/08/2020	09/09/2020	8
	Novel mix	10/09/2020	01/10/2020	16
Approximate 4-month break				
Rhino, main text	Indication	02/02/2021	05/02/2021	4
	30-sample discrimination	05/02/2021	18/02/2021	10
	60-sample discrimination	19/02/2021	26/02/2021	6
	100-sample discrimination	01/03/2021	08/03/2021	6
	Generalization/Retention Test	09/03/2021	23/03/2021	10
Approximate 5-week break				
Ivory, main text	Indication	05/05/2021	09/06/2021	24
	30-sample discrimination	10/06/2021	20/07/2021	24
	60-sample discrimination	22/07/2021	05/08/2021	11
	100-sample discrimination	06/08/2021	10/11/2021	67
	Glass vial test	11/11/2021	11/11/2021	1
	Generalization/Retention Test	12/11/2021	24/11/2021	9
Mixture tests, main text	Individual concealment	25/11/2021	29/11/2021	3
	Complex concealment	30/11/2021	07/12/2021	6

2   Detection of Concealed Wildlife Targets

Smugglers attempt to hide wildlife targets, selecting items that will conceal all aspects of the trafficked wildlife (*i.e.,* appearance and smell). Therefore, we explored whether rats ($n = 10$) would detect trained wildlife targets when they were encountered among non-target items and how much of the wildlife target needed to be present for the rat to detect it. These tests were carried out after the training and tests described in Experiment 1 of the main article.

Throughout the below described experiments, we encountered a variety of challenges related to apparatus function, rat motivation to complete sessions, and clerical errors. In short, we experienced a malfunction in the software of the apparatus described in the main manuscript, where rat indications and sniff duration of each sample were not recorded. As a result, several days of data were not usable. In addition, at the same time as we experienced a change in staff assigned to this project, there was a range of rats who did not complete sessions for a prolonged period, hence reducing the sample size and introducing inconsistency of which session was completed by which rat. Further, this was not always noted clearly on the records adding to the uncertainty of available data. Lastly, as experiments took place APOPO underwent a change in staff and supervision of the project experienced gaps during which not all data were extracted and stored as required. Due to the combination of these issues, the data were deemed unsuitable for statistical analyses; however, we present the methodologies used here for consideration of how these may have influenced rat learning or performance during the experiments and tests described in the main article. This serves to inform the reader on the type of training rats were exposed to during this period and provide context for the experiments carried out after, presented in the main text.

2.1   Method

We conducted initial tests and training with target mixtures containing varying mass ratios of Pangolin 1 and Wood targets mixed with a variety of non-targets (50:50, 25:75, and 10:90 target: non-target ratios). A fourth ratio (5:95) was introduced during the subsequent test.

2.1.1   Samples

Pangolin 1 and Wood served as targets as well as 10 non-target materials used during Experiment 1. Ratios were mixed as described in Supplementary Table 1 below.

Supplementary Table 2. Sample preparation list for mixtures of wildlife targets and non-target items at varying ratios.

Target:Non target mixture	50:50 ratio in gram	25:75 ratio in gram	10:90 ratio in gram	5:95 ratio in gram
Pangolin:Cable	0.5:4	0.25:6	0.1:7.2	0.05:7.6
Pangolin:Cardboard	0.5:0.5	0.25:0.75	0.1:0.9	0.05:0.95
Pangolin:Coffee	0.5:0.5	0.25:0.75	0.1:0.9	0.05:0.95
Pangolin:Dengu seeds	0.5:4	0.25:6	0.1:7.2	0.05:7.6
Pangolin:Peanut	0.5:0.5	0.25:0.75	0.1:0.9	0.05:0.95
Pangolin:Christmas tree seedpod	0.5:0.5	0.25:0.75	0.1:0.9	0.05:0.95
Pangolin:Christmas tree seed	0.5:4	0.25:6	0.1:7.2	0.05:7.6
Pangolin:Sock	0.5:0.5	0.25:0.75	0.1:0.9	0.05:0.95
Pangolin:Washing powder	0.5:0.2	0.25:0.3	0.1:0.36	0.05:0.38
Pangolin:Wig	0.5:0.5	0.25:0.75	0.1:0.9	0.05:0.95

Wood:Cable	0.5:4	0.75:6	0.3:7.2	0.15:7.6
Wood:Cardboard	0.5:0.5	0.75:0.75	0.3:0.9	0.15:0.95
Wood:Coffee	0.5:0.5	0.75:0.75	0.3:0.9	0.15:0.95
Wood:Dengu seeds	0.5:4	0.75:6	0.3:7.2	0.15:7.6
Wood:Peanut	0.5:0.5	0.75:0.75	0.3:0.9	0.15:0.95
Wood:Christmas tree seedpod	0.5:0.5	0.75:0.75	0.3:0.9	0.15:0.95
Wood:Christmas tree seed	0.5:4	0.75:6	0.3:7.2	0.15:7.6
Wood:Sock	0.5:0.5	0.75:0.75	0.3:0.9	0.15:0.95
Wood:Washing powder	0.5:0.2	0.75:0.3	0.3:0.36	0.15:0.38
Hardwood:Wig	0.5:0.5	0.75:0.75	0.3:0.9	0.15:0.95

2.1.2 Procedure

2.1.2.1 Pre-Tests

Three pre-tests were conducted to determine to which extent rats would spontaneously detect targets presented within mixtures. Each pre-test session involved 100 samples, of which 26 contained targets. Six target samples contained only the wildlife item (3 each of Pangolin 1 and Wood, 1 blind sample each), while the remaining 20 samples (10 each of Pangolin 1 and Wood) were mixed with non-target items at varying ratios (depending on the pre-test session, see below). The 74 non-targets included 29 isolated items and 45 mixed (varying ratios) of two non-target items. In between each pre-test, rats underwent two sessions of discrimination training identical to *Phase 3* of Experiment 1 in the main article.

Pre-Test 1 (n=2 sessions): Both target and non-target mixtures were prepared using a 50:50 ratio.

Pre-Test 2 (n=1 session): Pre-Test 1 revealed compromised detection accuracy for four mixtures (Pangolin 1 mixed with electrical cables, dengu seeds, washing powder, or synthetic wig) which remained at 50:50. Mixtures of all other samples were presented at a 25:75 ratio.

Pre-Test 3 (n=1 session): Mixture ratios were 10:90 Wood to non-target and 25:75 Pangolin 1 to non-target.

2.1.2.2 Concealed Target Training & Tests

Informed by the Pre-Test results, which suggested differences in rat performance based on specific mixtures and ratios, explicit training on "concealed targets" (targets presented mixed along with a non-target item) was conducted for the two targets sequentially, starting with Wood. A test was conducted at the end of each training phase, with five sessions of baseline training (identical to *Phase 3* of Experiment 1 in the main article) following each test.

Within each training session (regardless of the concealed target), 100 samples were presented, including 5 isolated targets (two Pangolin 1 and two Wood samples, one blind corresponding to the target undergoing concealed training) and 11 target mixtures (including one blind sample). Of 84 non-target samples presented, 70 were isolated and 14 were mixtures.

Target mixture ratios for specific mixtures were progressively reduced when ≥ 6 rats correctly detected the mixture during three out of five consecutive sessions. Therefore, within any session, rats could encounter a variety of mixture ratios depending on the specific target to non-target mixture.

Concealed Wood Training: Training began with 25:75 ratios. At the conclusion of training, sessions included 10:90 ratios of all non-target items except mixtures with peanuts and cardboard, which remained at 25:75.

Concealed Wood Tests: Ratios of 5:95 were presented during concealed target testing. During each session, we presented 16 samples with wildlife targets of which two were isolated Pangolin 1, and three were isolated Wood (1 blind). The remaining 11 target mixtures (1 blind) were with Wood. Non-targets were 70 pure and 14 mixtures. Target mixtures were counter-balanced across sessions to present each ratio (ranging from 50:50 to 5:95) twice across all sessions (Supplementary Table 2). Blind target mixtures never involved a novel ratio.

Concealed Pangolin 1 Training: After concealed Wood testing, rats underwent training on Pangolin 1, which began with 50:50 ratios. At the end of this training, sessions included 10:90 target mixtures for all non-targets except cardboard (25:75 ratio). Rats then advanced to concealed target testing.

Concealed Pangolin 1 Tests: This was the same protocol as described for *Concealed Wood Testing* but with Pangolin 1 as the main target instead of Wood.

Supplementary Table 3. Target mixture items and ratios presented during each test session. Target (either Wood or Pangolin) corresponded with the target for which mixture training had most recently finished.

Sample	Session 1	Session 2	Session 3	Session 4	Session 5	Session 6	Session 7	Session 8
Target:Peanut	50:50	25:75	10:90	05:95	50:50	25:75	10:90	05:95
Target:Electric cable	50:50	25:75	10:90	05:95	50:50	25:75	10:90	05:95
Target:Cardboard	25:75	10:90	05:95	50:50	25:75	10:90	05:95	50:50
Target:Coffee	25:75	10:90	05:95	50:50	25:75	10:90	05:95	50:50
Target:Dengu	10:90	05:95	50:50	25:75	10:90	05:95	50:50	25:75
Target:Pod	10:90	05:95	50:50	25:75	10:90	05:95	50:50	25:75
Target:Seed	05:95	50:50	25:75	10:90	05:95	50:50	25:75	10:90
Target:Cotton sock	05:95	50:50	25:75	10:90	05:95	50:50	25:75	10:90
Target:Washing powder	50:50	25:75	10:90	05:95	50:50	25:75	10:90	05:95
Target:Hair wig	50:50	25:75	10:90	05:95	50:50	25:75	10:90	05:95

3   Increasing Non-Target Variability

During the tests described under 1.1.2.2 we observed that rats committed an increased percentage of false alarms on novel non-target items. This strongly suggested that their detection behavior was driven by novelty seeking, rather than the smell of wildlife targets themselves. To mimic real-world scenarios, in which wildlife detection animals may encounter diverse and novel items daily, we presented the rats ($n = 9$) with a greater variety of non-target items. By increasing the variety of novel items encountered, this feasibility experiment aimed to train rats to adopt a target-seeking, rather than novelty-seeking strategy. In operational terms, this experiment assessed feasibility by assessing the likelihood of potentially costly false alarms to new (non-target) items.

The experiment below was carried out after the ratio tests described above and a subsequent seven-month pause in all training. Because all rats had previously been trained to detect the targets and a subset of non-target samples, two distinct training phases were adopted: Training 1 – Refresh

Training (training on original samples to re-establish baseline) and Training 2 – Novelty Training (training on a variety of novel non-target items to reduce apparent novelty-seeking behavior).

3.1 Method

3.1.1 Refresh Training

Indication Training (10th February 2020-20th February 2020): Only the first three odor holes of the apparatus were used during this stage, with each hole containing one of the familiar target samples (Pangolin 1 or Wood). Nine total samples (four or five of each target type) were randomly positioned within three bars. Rats advanced from indication training when eight of nine rats indicated ≥7 targets during two consecutive sessions.

Phase One (21st February 2020-2nd March 2020): Rats were presented with 30 samples, including ten targets (five of each target type) and 20 non-targets (two of each item from Experiment 1, see main text) randomly positioned per session. During the first two sessions, rats only had access to the first three holes of the apparatus. No more than two targets could occur within the same set of 3-samples and no more than two bars could contain only non-targets. Beginning with the third session, samples were positioned in all ten holes of the apparatus. When at least seven (of nine) rats hit ≥ 8 targets and committed no more than five false alarms, we introduced non-reinforced blinds (one of each target type).

Phase Two (3rd March 2020-27th March 2020): Rats were presented with 100 samples per session, including ten target samples (same as above) and 90 non-target samples (nine samples of each non-target item).

3.1.2 Novelty Training

As with Refresh Training, rats were presented with 100 samples per session, including ten target samples (two blinds) and 90 non-targets. Training was separated into two stages taking place from 30th March 2020-17th August 2020).

Phase I – increasing the number of non-target items: Both targets (Pangolin 1 and Wood) were presented five times per session. Non-target samples changed weekly. During the first week of training, six of the ten non-target items presented previously (familiar items) were randomly selected (seven or eight samples of each per session), while the remaining 45 samples were comprised of six completely new materials. Total samples per novel item was randomized daily. After one week of training, novel items were added to the library of familiar items and a new set of novel items was introduced. This effectively increased variety in all non-target items encountered across weeks and continued for the first seven weeks.

For the following two weeks of this phase (weeks 8-9), familiar items increased from six to nine with six samples each (54 total), while maintaining six novel items (36 total) per session. Two of the six novel samples were required to have assumed biological relevance (e.g., food pellets or conspecific bedding) or be items which generated more false alarms. During week 10, the number of familiar items increased to 12 (five samples of each item).

Phase II - increasing the number of non-target items and target specimens: After completing ten weeks of *Phase I* training, samples from two additional pangolin specimens were added as targets

(Pangolin 2 and 3). Rats had encountered Pangolin 2 during the Generalization Test of Experiment 1 described in the main text. Pangolin 3 was comprised of four individual scales provided to APOPO by the Endangered Wildlife Trust, South Africa.

Sessions continued to include ten targets, comprised of two Wood and two of each Pangolin specimen. One Wood and one randomly selected Pangolin sample served as blinds (but Pangolin 2 and 3 were only included as blinds after the first three sessions of training). Of the 90 non-target samples, 30 were novel during the first three weeks of this phase with six different non-target types. During weeks 4-8 of this phase, only six novel non-targets (one type) were presented per session alongside 14 familiar non-target types. These non-targets changed each week.

4   Discussion

The tests presented within these supplementary materials were carried out to offer insight into rats' ability to detect small concentrations of wildlife targets when concealed among non-target items.

Following the prolonged training on novel non-target items, rats showed an overall low percentage of false alarms, while consistently continuing to indicate wildlife targets. To ensure these results were driven by a target-seeking rather than a novelty-rejecting strategy, additional tests with novel mixtures of both targets and non-targets were conducted (please refer to the mixture tests presented in the main article).